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## PROGNOSIS IN HODGKIN'S DISEASE

By DORTHE ROOS and AAGE VIDEBÆK

The object of the present study was to ascertain whether the survival in Hodgkin's disease has been prolonged during the past decade.

In the middle of the forties, cytotoxic agents were introduced in the treatment of Hodgkin's disease, primarily nitrogen mustard (Erasol) and chlornaphazium (INN) (Erysan, Cloronaftina) as suggested by Gilman & Philips (7), Goodman et al. (8). Other agents that have been used are tretamine (TEM) (Philips & Thiersch (29), Rose et al. (30)), phenylbutazone (Krauss (18)), and 6-mercaptopurine (Rhoads (29)).

It was soon apparent that the cytotoxic agents were indicated only in the generalized forms of Hodgkin's disease, whereas its localized stages still responded better to X-rays (1, 6, 13, 16, 34). In part, the remissions obtained with cytotoxic agents were shorter and in part the various chemotherapeutics exert general cytotoxicity, primarily depressing the sensitive haemopoiesis as emphasized by Bichel (2) and by Hansen & Bichel (11).

Furthermore, it must be borne in mind that during the past decade antibiotics have been available in the management of the secondary infections, such as bronchopneumonia, while ACTH and corticosteroids have been used in controlling bone-marrow damage, hæmolytic states, and febrile periods.

## MATERIAL

The analysis was based on the comparison of two groups of patients.

*Group I* comprises 172 patients (90 males and 82 females) treated as out-patients or in-patients at the Radium Centre, Copenhagen, during the period 1930—45. Prognostic studies in this group of cases were published by one of us in 1950 (35).

*Group II* comprises 117 patients (59 males and 58 females) derived from the period 1946—55, also treated at the Radium Centre.

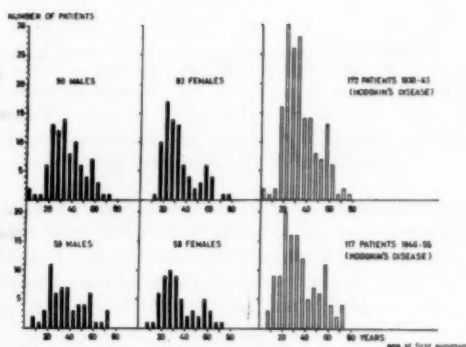


Fig. 1.

Age distribution curves for males and females from the two groups of patients, by age at appearance of first symptom.

On Fig. 1 the patients are grouped by age at the onset of the first symptom as far as it could be ascertained from the case records of the Radium Centre or data obtained elsewhere\*).

The sex ratio in the two groups of the present series is approximately the same: 52 per cent and 50 per cent males, or a relatively lower male incidence than in most other materials. Slaughter & Craver (32) reported 62 per cent, Jackson & Parker (14) 70 per cent, Peters (27) 66 per cent, Paterson & Paterson (26) 66 per cent, Hall & Olsson (10) 69 per cent, O'Brien & O'Brien (24) 75 per cent, Jelliffe & Thomson (15) 61 per cent, Clemmesen (4) 57 per cent, while Loew (20) had only 42 per cent males. As for women of the fertile age (15—45 years) the two groups included 64 (37 per cent) and 41 (35 per cent). The average age at the onset of the first symptom was 34.1 years in Group I (34.2 years for males and 34.0 years for females) and 35.9 years for Group II (males: 37.3 years and females 34.5 years). This difference

\*) Without the data very kindly supplied by hospital physicians as well as general practitioners this analysis would not have been possible.

PERCENTAGE  
of patients (Hodgkin's disease)

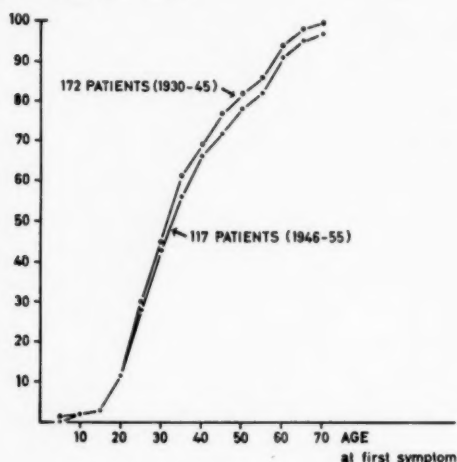


Fig. 2.

Probit age distribution curves for the two groups of patients with Hodgkin's disease.

can hardly mask a longer survival time, because in fact the survival time fluctuates but little with the age at the first symptom. Videbæk (35), for instance, reported a difference of two months in the survival time of the 10–20-year-group compared with the 20–30-year-group, *i. e.*, a variation of 5 per cent for a decade.

Lastly, the probit age distribution curves for both groups are plotted on Fig. 2. The two curves deviate but little, so the two groups must be directly comparable.

**Diagnosis.** This was confirmed by histological examination of biopsy specimens in all cases and in autopsied cases also by the post-mortem findings. In Group I post-mortem data are available for 46 (= 32 per cent of those possible) and in Group II for 48 (= 55 per cent of those possible). No attempt was made to classify the cases, according to the histological findings, into Hodgkin's paraganuloma, Hodgkin's granuloma, and Hodgkin's sarcoma (14). This could not be done for several reasons: The histological degree of malignancy is not always stated, the microscopical investigations have not always been carried out by the same histologist, and finally there may, at a certain time, be transitions between the different stages of malignancy, even in the same lymph node.

#### TREATMENT

In Group I the patients had been treated exclusively by X-rays and possibly blood transfusions. As a rule the dosage of X-rays was 100–200 r in series of 10–20 sittings (depending upon the region, response, and tolerance),  $\frac{1}{2}$  mm Cu, focal-skin distance 40–60 cm.

In Group II the patients had received, in addition to blood transfusions and X-rays in the above-

mentioned doses, also cytotoxic agents, antibiotics, ACTH or corticosteroids, depending on the generalization of the disease, the patient's general condition, blood picture, and complications, if present. The majority, or 82 (70 per cent) were treated by X-rays as well as cytotoxic agents, 31 (26 per cent) only by X-rays, one only by nitrogen mustard, while one had been treated by splenectomy in addition to the chemotherapy and one exclusively by surgery (removal of the lateral cervical lymph nodes). Seventy-eight (67 per cent) had chloroethyl amines, either in the form of nitrogen mustard in doses of 0.05–0.1 mg per kg body weight in series of 6–8 *i. v.* injections, or in the form of chlornaphazium, generally in doses ranging from 100 to 400 mg daily for several months, depending on tolerance, severity of the disease, and blood picture.

Phenylbutazone was administered to 11 patients (10 per cent) (100–400 mg daily for some months) and TEM to five (4 per cent) (5 mg every other day for about one week at a time).

Fifty-nine patients (50 per cent) received antibiotics, usually penicillin, for controlling intercurrent infections.

Forty-three patients (37 per cent) received ACTH, cortisone, or prednisone, usually because of haemolysis, leucocytopenia, or fever and itching which had proved intractable by other means.

#### PROGNOSIS

The prognosis was evaluated on the basis of the survival time, calculated from the first symptom. For all cases there is a minimum follow-up period of 3 years. At the time of the study 29 (17 per cent) of Group I and 30 (26 per cent) of Group II were alive.

As suggested by Hall & Olsson (9) the series were analysed according to the general principle within mortality statistics: The number of patients dying within a given interval after the onset of the disease was divided by the number of patients who passed this interval or more correctly the number of years that the patients have survived altogether during this interval (*cf. e. g.* Nyholm & Helweg-Larsen (23)).

Tables 1 and 2 set out the patients of the two groups according to this principle which has shown reliable figures for three-, five- and seven-year survival times in similar materials including surviving patients with a short follow-up period.

Years of survival (second column) indicates the number of years that the total cases have survived during the period concerned (first column).

Division of the years of survival into the number of deaths during the interval (third column) gives the death intensity (fourth column). On this basis the survival rate may be calculated (fifth column,  $1_x$ ) which gives the percentage of patients surviving after *x* years.

Table 1.  
Survival table for 172 patients with Hodgkin's disease whose disease set in during the period 1930-45.

Duration after onset of first symptom (years)	Years of survival	Number of deaths	Death intensity	Survival rate (in per cent) $I_x$	S.D.
0	163.6	21	.128	100	
1	136.0	32	.235	88.0	
2	105.2	30	.285	69.6	
3	75.0	18	.240	52.3 $\pm$ 3.8	
4	55.9	13	.233	41.1	
5	39.8	10	.251	32.6 $\pm$ 3.7	
6	27.8	6	.216	25.4	
7	18.7	6	.321	20.4 $\pm$ 3.3	
8				14.8	

Table 2.  
Survival table for 117 patients with Hodgkin's disease whose disease set in during the period 1946-55.

Duration after onset of first symptom (years)	Years of survival	Number of deaths	Death intensity	Survival rate (in per cent) $I_x$	S.D.
0	112.3	16	.142	100	
1	92.9	14	.151	86.6	
2	79.8	14	.175	74.6	
3	68.6	10	.146	62.6 $\pm$ 4.5	
4	51.8	14	.270	54.1	
5	36.7	6	.163	41.3 $\pm$ 4.6	
6	27.8	2	.072	35.1	
7	19.7	6	.305	32.7 $\pm$ 4.6	
8				24.1	

For the survival periods 3, 5, and 7 years  $\pm$  the standard deviation of  $I_x$  is given, but for the latter period the calculations are of course less reliable than for the first ones.

On Fig. 3 the survival curves of the two groups are plotted with the time as the abscissa. It is evident that the curve for the 1946-55 period (Group II) is considerably higher than for the 1930-45 series (Group I).

Table 3.  
Statistical comparison between survival rate for 172 patients with Hodgkin's disease which had set in during the period 1930-45 and 117 patients with Hodgkin's disease which had set in during the period 1946 and 1955. 3, 5, and 7 years after the appearance of the first symptom.

Duration	3 years			5 years			7 years		
	$I_x$	diff.	S.D.	$I_x$	diff.	S.D.	$I_x$	diff.	S.D.
Group I (1930-45)	52.3			32.6			20.4		
Group II (1946-55)	62.6	-10.3	(5.8)	41.3	-8.7	(5.9)	32.7	-12.3	(5.6)

SURVIVORSHIP  
PERCENTAGE (Hodgkin's disease)

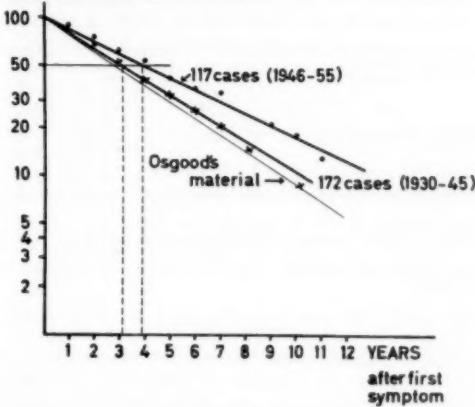


Fig. 3.  
Survival curves for the two groups of patients with Hodgkin's disease. The bottom curve represents the survival curve on the basis of Osgood's collected material from the literature.

Table 3 presents the statistical comparison. When the difference between  $I_x$  for Groups I and II exceeds twice the standard deviation, calculated according to

$$\sqrt{SD_I^2 + SD_{II}^2}$$

it may be considered significant in respect to the 5 per cent limit. This is true of the 7-year period, and the figure for the 3- and 5-year periods tend into this direction.

On Fig. 3 we have also plotted a curve representing 650 patients, recently published by Osgood (25) on the basis of Shimkin's (31), Stout's (33), and Nathanson & Welch's (21) prognostic analyses. Like our patients of Group I, Osgood's had received no specific therapy except X-rays.

The difference between the 1946-55 group from the Radium Centre and Osgood's series is even more marked than that between the two Danish series. Table 4 shows a more favourable prognosis for all durations, and this finding is statistically significant with respect to the five per cent limit. In the case of this comparison the standard deviation is moreover probably a maxi-

Table 4.

Statistical comparison between survival rate for 650 patients with Hodgkin's disease (Osgood's material) treated with X-rays alone and 117 patients with Hodgkin's disease treated at the Radium Centre, Copenhagen, during the period 1946-55. 3, 5, and 7 years after the onset of the first symptom.

Duration	3 years			5 years			7 years		
	1x	diff.	S.D.	1x	diff.	S.D.	1x	diff.	S.D.
Group II (1946-55)	62.6			41.3			32.7		
Osgood	48.2	14.4	(4.5)	27.8	13.5	(4.6)	17.0	15.7	(4.6)

imum value, since no regard was paid to the uncertainty attaching to Osgood's series (not stated).

#### DISCUSSION

The reported prognostic analyses include only a few patients treated exclusively by chemotherapy. If so, these are generally patients who have had generalized Hodgkin's disease when first seen, *i. e.* Peters' (27) Group 3, patients in whom at least two separate groups of lymph nodes are involved and who present generalized signs, such as fever, anorexia, itching, etc. As shown by previous authors (*e. g.* 8, 12, 15, 20, 22, 27), such patients have a poorer prognosis, and any comparison between various forms of treatment must presuppose that a possible prognostic difference is not distorted by factors which influence the prognosis at all, such as age (poor prognosis for patients of advanced age (15, 17, 35)), sex (better prognosis for women of the fertile age (3, 5, 15)), or the spread of the disease (*vide supra*).

The most important prognostic factor is without doubt the propagation of the disease at the onset of the first symptom. Both groups of patients are derived from the Radium Centre, Copenhagen, which receives patients for out-patient treatment or in-patients from general practitioners or other hospitals, and there is no reason to presume any

shift between benign and more malignant cases during the past decade. Originally, Group II comprised, in addition to the 117 patients from the Radium Centre, also 26 patients from Rigshospitalet, Medical Department A. These 26 patients were excluded, however, partly because their average age (37.3 years) was higher than that of the patients from the Radium Centre and partly because most of these "medical" patients' disease did not manifest itself until it had become generalized. These patients were admitted to the University Hospital because of systemic signs, such as fever, itching, sweating, and anaemia. This type invariably has a more serious prognosis as emphasized by Iversen & Kjerulf (13) as early as 1948.

Gellhorn & Collins (6), in 1951, found no statistical significance to indicate that chloroethyl amines prolonged the survival time. Analysing a material from the University of California, Shimkin et al. (31) arrived at the same result in 1955.

Hall & Olsson (10), treating 31 patients by chemotherapy and X-rays and another 31 by X-rays alone, found no significant difference in the survival time. In a comprehensive study of the prognosis in Hodgkin's disease (25) Osgood analysed these results and found no significant difference in the prognosis of the two groups.

Table 5.

Results of various prognostic studies. All survival times calculated from the onset of first symptom. The letter in the last column indicates which of the three named requirements has been observed in obtaining the results (*cf. the text*).

	Year	3-year survival in per cent	5-years survival in per cent	No. of patients	Method and comments
Nathanson & Welch	1937	43	23	184	b
Slaughter & Craver	1942		29	265	b
Jackson & Parker	1947		22	231	b (rep. by Osgood)
Stout	1949	53	23	212	b (rep. by Osgood)
Videbæk (analysed 1958)	1950	52.3	33	172	c
Shimkin et al.	1955	47.8	26	230	b
Jeliffe and Thomson	1955		29	172	b
Healy, Amory & Friedmann	1955		44	216	b (military series)
Loew	1955		57	65	b (small series, mainly women)
Paterson & Paterson	1955		25	256	b
Hall & Olson (analysed in 1958)	1956	51	28	62	c
Roos & Videbæk	1958	62.6	41.3	117	c



The most positive attitude to early chemotherapy, also in the localized type, has been exhibited by Larionov (19) who advocates intermittent chemotherapy and X-radiation as the primary treatment. After this form of treatment he had a five-year survival rate of 33 per cent, but the sex ratio and age distribution are not stated, and the series may have been selected.

Thus, our results go against those of previously published prognostic studies. At the same time, however, it seems justified to stress that it would not be correct to give the cytotoxic agents all the credit for the improved prognosis. During the past decade, the treatment has also been supplemented by antibiotics, corticosteroids, and more frequent blood transfusions.

#### NUMERICAL COMPARISON WITH OTHER PROGNOSTIC STUDIES

Comparison between the survival times in different series involves many sources of error (25). Such comparisons are necessary (Table 5), however, in order to gain an impression of the possible prognostic influence of a treatment. The following requirements should be fulfilled:

- (a) the series must be total (*i. e.* all the patients have died at the time of the analysis), or
- (b) the stated survival time must not exceed the follow-up period, or
- (c) the survival time must have been found by a statistically reliable calculation.

Table 5 sets out various prognostic analyses. All survival times are calculated from the onset of the first symptom. Healy et al.'s analysis from 1955 is based on a military series, so it does not carry the more serious prognosis of older age groups. Loew's five-year survival must also be considered with a certain reserve, since his series is small and comprises mainly women.

#### SUMMARY

In an effort to ascertain whether modern therapy has improved the prognosis in Hodgkin's disease, the authors compared the survival curve for 172 patients (Group I) whose disease set in during the period 1930—45 with that for 117 patients (Group II) who had their first symptom during the period 1946—55.

It is demonstrated that the groups of patients are comparable. While the patients of Group I were treated with X-rays alone, Group II also received cytotoxic agents, corticosteroids, and antibiotics.

The five-year survival rate is 33 per cent for Group I and 41 per cent for Group II. The prognostic improvement is statistically significant.

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## INDICATIONS FOR OPERATION IN ULCERATIVE COLITIS

By JOHN LINDENBERG

Surgical treatment of ulcerative colitis consists most frequently of establishment of an ileostomy. According to the individual needs, this may be supplemented later by subtotal colectomy and removal of the rectum.

This paper, based upon 74 cases submitted to surgical treatment, is designed to account for the most common indications for operation.

## INDICATIONS FOR OPERATION

Operation may be indicated in the following groups of patients:

- 1) patients with regional ulcerative colitis;
- 2) patients suffering from complications of ulcerative colitis;
- 3) patients suffering from uncomplicated ulcerative colitis.

Quite good agreement has been attained during the course of years concerning the first two groups as regards the attitude towards operation, but the tendency to operate in uncomplicated cases too has increased in recent years.

1) Regional ulcerative colitis, even when associated with mild clinical symptoms only, should be submitted to operation if it neither undergoes remission nor reacts to conservative therapy and before the pathological processes descend to the sigmoid colon (Chron et al. 1938, Dennis 1945, Stone 1951).

2) Complications of ulcerative colitis occur in  $\frac{1}{3}$  to  $\frac{2}{3}$  of the patients, are frequently multiple and increase in frequency with the severity and the duration of the disease (Ricketts et al. 1946, Kirsner et al. 1948). Complications comprise: a) complications from the colon, b) complications from adjoining structures and c) distant complications.

a) *Complications from the colon.* Pseudopolyposis and polyposis must be regarded as premalignant conditions and are, therefore, indications for operation (Cattell 1948, Ferguson et al. 1948, McKittrick et al. 1949). These conditions are frequently encountered after the clinical symptoms have disappeared. The polypi may be kept under observation for a brief period if conditions permit (Gabriel 1952), as pseudopolyposis may disappear spontaneously (Counsell et al. 1951/52).

Cancer of the colon is a rather frequent complication in cases of ulcerative colitis, especially in young patients (Hickey et al. 1958, Dukes

1958) and is naturally an indication for operation, but the lesion is frequently an adenocarcinoma of high malignancy (Counsell et al. 1951/52, Shands 1952) and the period of survival after the development of the carcinoma is, therefore, brief. In individual materials, all of the patients with macroscopically recognized cancer died within two years (Lahey 1950, 1951) and cancer complicated by obstruction is, as a rule, inoperable (Lahey 1950).

Stricture of the colon is an indication for operation. It may not only cause intestinal obstruction but, in addition, a risk exists of haemorrhage and perforation and of the development of polypi proximal to the stricture (Brown et al. 1950, Cattell 1953).

Massive colonic haemorrhage is, similarly, an indication for operation as it indicates deep ulceration in the colon. Such cases often run an acute course and the prognosis is poor (Dennis 1945, McKittrick et al. 1949, Palmer 1948).

b) *Complications from adjoining structures.* Perforation may occur during an acute fulminant course and in connection with massive haemorrhage from the colon. This is the most serious of the acute complications. The perforations are frequently multiple and the lethality is 75–100 per cent (Jankelson et al. 1945). Impending perforation and acute perforation into the free peritoneal cavity must, therefore, be regarded as indications for operation (Kirshen 1949, Patterson 1951). Some surgeons refrain from operation when manifest peritonitis is present (MacKenzie 1951). The operative treatment of perirectal and perineal complications with abscess and fistula formation is particularly important. These complications must be treated locally with drainage of the abscesses and also by ileostomy (Cattell 1953).

c) *Distant complications.* Arthritis is often associated with ulcerative colitis (Bywaters et al. 1958). It is possibly connected by an allergic mechanism to the intestinal condition (Rice-Oxley et al. 1950) and indicates operation as chronic joint changes may otherwise develop. Operation should be undertaken before the joint surfaces are damaged (Cattell 1953, Jones 1953).

Skin complications which occur probably on account of disturbances of nutrition may be indications for operation with ileostomy. The most serious is phagedena geometricum (Russell

1950). This disease may commence following furunculosis, trauma following injection or erythema nodosum with the development of extensive skin lesions. Such violent affections may occasionally develop that amputation of extremities may be necessary to save life. (Butler 1950, Jankelson et al. 1936).

Rheumatic eye complications may be indications for operative treatment (Jones 1953) and, similarly, damage of the parenchymatous organs such as commencing liver injury. If the damage to the liver is fully developed the prognosis at operation is poor (Jones 1951). Endocrine disturbances which may be present in children with the development of infantilism (Corbett 1944/45) may also be indications for operation.

3) Uncomplicated ulcerative colitis is now regarded by the majority of authors as an indication for operation, when medical treatment has proved ineffective (Cave 1958, Dennis 1945, Garlock 1945/46, Kirsner 1957, Palmer 1948).

Special insight into the clinical picture in the individual patient is necessary to establish the indications for operation and in this connection, three factors may guide the choice: 1) the pathological changes in the colon, 2) the symptoms alone and 3) the social circumstances.

Increasing progression of the pathological processes in the colon is frequently encountered following repeated episodes of deterioration in the disease, in which the course of the disease shows a steady although slow deterioration. These patients should be submitted to operation before the entire colon is affected (Jones 1938, Cave 1945) as they are frequently just kept alive as chronic invalids by continuous medical treatment.

General symptoms of the disease alone may demand surgical treatment. Thus, persistent intestinal haemorrhage, even if not of the character of massive haemorrhage, may occasion intractable anaemia which resists even transfusion therapy and therefore may be an indication for operation (McKittrick et al. 1935, Cattell 1953, Cave et al. 1938). Defective nutrition may be a decisive indication for operation (Cave et al. 1938, Emery et al. 1934). Persistent pyrexia, particularly septic temperature with raised pulse rate, frequently suggesting a local infective complication, may, similarly, indicate operation (Lahey 1931, McKittrick et al. 1935) and abdominal pain alone may be so incapacitating that operation is necessary. This holds also true for persistent diarrhoea. Even general dyspeptic complaints such as nausea, anorexia and vomiting, particularly when these are associated with low serum protein, may be indications for operation on account of threatened disturbances of nutrition.

During recent years, the social circumstances have been taken into consideration to an increasing extent (Ault 1949, Best 1947, Brown et al. 1950, Cave 1958, Colcock 1950, Froese 1951, Ripstein et al. 1952). It has become recognized that intractable semi-invalids should not continue under medical control but that they should be relieved of their symptoms by surgical intervention.

To illustrate this question, 67 patients treated solely by medical means were reviewed. The material comprises 24 males and 43 females who were, on an average, 30 years of age at the commencement of the disease; 46 per cent had an acute commencement. The medical treatment employed consisted of enemata, chemotherapy, antibiotics, blood transfusions, iron and liver preparations, vitamins and in few cases ACTH and cortisone. No rapid effect attributable to the treatment was observed in any case. The lethality in the course of the disease was 30 per cent (20 patients out of 67 died) and the duration of the disease in the patients who died was, on an average, 5.3 years. The average age at death was 38 years and out of the entire material of 67 patients 21 died prior to the age of 40 years. Follow-up examination involved 47 patients with an average period of observation of 15 years. Out of the original material with 30 per cent deaths, 19 per cent were fit for work and 51 per cent unfit for work or only partially fit for work at the time of the examination. The longer the period of observation, the greater the percentage of the patients in whom the capacity for work was reduced as the result of symptoms of ulcerative colitis (Fig. 1).

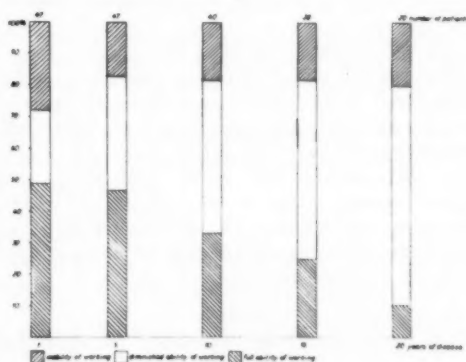


Fig. 1.  
Working capacity of the patients during the course of disease. (The percentage alteration in the working capacity during the period of observation).

#### SURGICAL MATERIAL

The indications for operation reviewed above were employed as a basis for operative intervention in a material of 74 patients. The average age of these patients was 28 years at the commencement of the disease. The material comprises

Table 1.

*Operations performed subsequent to ileostomy, and number of deaths in relation to these.*

74 pts. with ileostomy	11 pts. dead	(15%)	6 pts. dead	(13%)	2 pts. dead	(9%)
	48 pts. subtotal colectomy	(65%)	22 pts. removal of rectum	(45%)	20 pts. survived	(91%)
	13 pts. without further operation	(17%)	20 pts. without operation	(42%)		
	2 pts. total colectomy	(3%)				

35 males and 39 females and in 76 per cent of the cases, the disease had commenced acutely.

#### Treatment.

In all of the cases, the patients were submitted to operation following medical treatment. Operation consisted of ileostomy and, if necessary, this was later supplemented by colectomy and removal of the rectum. The distribution of operation is shown in Table 1 which shows that in 32 per cent of the patients ileostomy alone was undertaken during the period of observation, in 68 per cent subtotal colectomy was undertaken in addition and in 41 per cent the entire colon and rectum were extirpated.

Table 2.

*Main indications for ileostomy, subtotal colectomy and removal of the rectum in 74 patients, expressed in per cent.*

	Number of Patients	Serious Complications	Serious Deterioration of Symptoms	Chronic Course
Ileostomy	74	34	35	31
Subtotal Colectomy	50	33		67
Removal of the Rectum	22	41		59

#### Main indications for operation.

The main indications for operation appear from Table 2. In 65 per cent of these ileostomy patients subtotal colectomy was later undertaken and in 30 per cent the entire colon and rectum were extirpated. The main indications were serious complications in 33 and 41 per cent of the cases, respectively, the remainder were submitted to operation on account of clinical symptoms.

Table 3.

*Complications as main indications for ileostomy (25 patients)*

<i>Complications in colon:</i>	
Pseudopolypoidosis	3 patients
Massive haemorrhage	3 patients (1 dead)
<i>Complications in adjoining structures:</i>	
Intestinal perforation	3 patients (1 dead)
Impending perforation	5 patients (2 dead)
Perineal reaction	5 patients (2 dead)
<i>Distant complications:</i>	
Skin complications	4 patients
Arthritis	2 patients

The complications present prior to the ileostomy operation appear from Table 3, which also shows that when complications were the main indications for operation the lethality was 24 per cent and that deaths only occurred in connection with haemorrhage and perforation of the intestine.

Table 4.

*Influence of ileostomy on preoperative symptoms, expressed in per cent*

	Patients (Number)	Ceased	Abated	Unchanged	Deficient Information
Diarrhoea	60	82	8	3	7
Pain	46	59	28	7	6
Haemorrhage	45	27	29	38	6
Temperature rise	46	85	2	11	2
Pulse over 120/min.	47	0	55	41	4

#### Results.

Clinical symptoms which were present prior to the ileostomy operation and their reaction to the operative intervention are indicated in Table 4. In the overwhelming majority of the cases, operation produced immediate cessation or abatement of the symptoms but only in 18 per cent of all the patients did ileostomy suffice during the period of observation, indicating the tendency to recurrence in the course of time. To the symptoms mentioned, may be added anaemia, low serum protein and oedema as particularly frequent. These symptoms are not recorded in the table because treatment with blood transfusion both pre- and post-operatively was employed. Nor are loss of weight or failure to gain weight recorded but these symptoms were present in 62 per cent of the cases. In the majority of cases, following a preliminary loss of weight immediately after operation the patients improved in this respect and in 50 per cent of the patients weight increased during the months immediately after operation.

#### Mortality from the primary operations.

The mortality appears from Table 1. It is practically identical for ileostomy (15 per cent) and subtotal colectomy (13 per cent) and slightly less for removal of the rectum (9 per cent). The total mortality from operation was 26 per cent and the mortality for the ileostomy operation when the main indication was an acute complica-



tion was 27 per cent, in cases of acute deterioration of the disease 13 per cent and from operation undertaken during the course of chronic disease 9 per cent only.

# Follow-up Investigation.

The follow-up investigation involves 54 patients with an average period of observation of 4½ years. None of the patients were fit for work prior to operation. The mortality and the ability to work at the follow-up examination appears from Table 5. All of the patients followed-up who survived the operations and who were not recently discharged from hospital were fit for work on follow-up investigation. This comprises 65 per cent of all the patients.

Table 5.

Distribution of the surgical patients with regard to mortality and working capacity at the follow-up, expressed numerically and in per cent.

55 patients survived operation	42 patients fit to work (follow-up)	65 %
	6 patients fit to work, according to own doctor	
	2 patients fit to work on last examination or symptom- free, but disabled at follow- up	
	1 patient just discharged, symptom-free	
	3 patients still in hospital	4 %
	1 patient died 6 years after operation	1 %
	19 patients died postoperatively	26 %

## DISCUSSION

When the indications for operation in ulcerative colitis are considered, various conditions should be evaluated:

1) The result of medical treatment has remained unchanged throughout a period of more than 30 years as regards the mortality, which for extensive groups of patients followed throughout a series of years lies between 30 and 33 per cent (Brown et al. 1951, Kapel 1950, Moltke 1935, Rice-Oxley et al. 1950).

2) The mortality from the surgical treatment has fallen to a level which, in any case, does not exceed that from medical treatment. In the author's material, the operative mortality was thus 26 per cent while the mortality for patients treated medically was 30 per cent.

3) With medical treatment 70 per cent and up to 90 per cent of the patients are stated to be improved or cured when the treatment considered best at the time was employed (Chron et al. 1924, Streicher et al. 1930, Best 1938, Svartz 1946, Strax et al. 1951), but only brief periods of observation were involved. Patients who are followed-up over a series of years from the commencement of the disease, present re-

currence in 90 per cent of cases (Rice-Oxley et al. 1950).

4) While all patients following ileostomy and subsequent operations become fit for work following a suitable period of convalescence, in the medical materials up to 50 per cent of the patients will be found to be unfit for work or only partially fit for work and the longer the period of observation the greater will be the percentage of patients with reduced capacity for work.

Operative therapy, therefore, when undertaken on correct indications and at the correct time, does not expose the patient to greater risk as regards mortality than prolonged medical treatment and it will enable the patient to attain a more normal productive state. In order not to expose the patients to unnecessary risks at operation, this should be undertaken before acute complications arise and before chronic complications have produced irreparable injury such as arthritis and the development of cancer. The operative interventions should, thus, be carried out as elective operations.

## SUMMARY AND CONCLUSIONS

A material of 74 cases of ulcerative colitis treated surgically has been presented.

The main indications for surgical treatment in ulcerative colitis may be tabulated as absolute and relative indications.

1) *Absolute Indications*: a) Regional ulcerative colitis, b) ulcerative colitis with a more complicated course: polyposis, colonic stricture, massive colonic haemorrhage, perforation of the colon, certain skin and eye complications, incipient parenchymatous damage and arthritis, c) ulcerative colitis with particularly severe general symptoms: diarrhoea, persistent intestinal haemorrhage, loss of weight, pain, highly febrile or septic temperature and a pulse rate exceeding 120/min.

2) *Relative Indications*: These are present in patients who are unfit for work on account of symptoms of ulcerative colitis provoked, *inter alia*, by work.

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## ILEOSTOMY DYSFUNCTION AND ITS PREVENTION

By JOHN LINDENBERG

Transverse ileostomy was introduced by Brown in 1913 and has been employed to an increasing extent in intestinal surgery primarily in the treatment of ulcerative colitis and multiple polyposis of the colon.

The increasing employment of ileostomy operations has been rendered possible following the introduction of the Koenig-Rutzen ileostomy bag, which *inter alia* successfully protects the skin from the potent erosive action of intestinal secretions. In addition, the indications for operation, particularly in ulcerative colitis, have been extended during the past ten years and the mortality following ileostomy has fallen to 1/4—1/5 of that previously found, *viz.*, from approximately

20 per cent to approximately 4 per cent (Lahey 1950, Cattell 1953).

Prior to 1946, ileostomy dysfunction was only mentioned by isolated authors (Cave et al. 1940, Welch et al. 1937) but deaths attributable to ileostomy diarrhoea were described (Barger et al. 1943). During recent years, however, several authors have concerned themselves with the problem (Colcock 1951, Fahy et al. 1957, Fleischner et al. 1958, Ferguson et al. 1947, Garlock et al. 1957, Lahey 1950, Niederle 1956, Rapoport 1956, Turnbull 1953, 1956 and Warren et al. 1951).

### CLINICAL PICTURE

Slighter cases are characterized by spasmodic abdominal pain during the days immediately after operation and the discharge from the ileostomy remains watery and of a greenish brown

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colour and is increased in quantity. In a fasting adult patient with an ileostomy the daily volume of the ileostomy discharge is approximately 500 ml. Discharge of over one litre daily should arouse suspicion of commencing obstruction (Warren et al. 1951). The more severe cases are characterized by marked colicky abdominal pain most frequently localized around the ileostomy, and the watery discharge from the ileostomy may increase in volume to several litres daily. The abdomen becomes distended, nausea and vomiting develop, dehydration increases and signs of electrolyte loss occur, manifesting itself in a shock-like state. In the more advanced stages, pain may cease (Warren et al. 1951). Only in very rare cases does the discharge from the ileostomy cease. The signs and symptoms of ileostomy dysfunction usually respond to suitable treatment during the first four weeks after operation.

These patients thus present the clinical picture of obstruction of the small intestine and X-ray examination will reveal signs of intestinal obstruction with distension of the intestine and fluid levels.

#### AETIOLOGY

The cause of post-operative ileostomy dysfunction is always localized to the opening of the ileostomy or its immediate surroundings. This consist of intermittent obstruction of the lumen of the intestine and may be caused by oedema of the intestine (Frobese 1951, Lahey 1950), by adhesions, torsion of the ileum or oedema of the abdominal wall (Colcock 1950, 1951), by inflammation with abscess formation in the abdominal wall around the intestine or too tight closure of the abdominal wall, particularly the peritoneum, around the intestine (Ferguson et al. 1947). The most important cause of dysfunction may be changes in the stump of the ileum outside the skin (Turnbull 1953, Warren et al. 1951). A few days after operation, fibropurulent patchings and induration of the peritoneum occur. The intestine becomes increasingly rigid and peristalsis diminishes. Gradually, scar tissue forms on the outer surface of the intestine and the mucosa is everted towards the edge of the skin.

#### TREATMENT

Treatment is primarily conservative with digital dilatation of the ileostomy if the only symptom is colicky abdominal pain (Warren et al. 1951). If the symptoms are more severe, catheterization of the ileostomy for periods of several hours at a time is recommended. Some surgeons employ a cuffed indwelling catheter which is introduced until recovery from the episode of dysfunction occurs (Turnbull 1953). This method, however, involves a risk of perforation of the intestine.



Fig. 1.

*The operation performed; the midline incision in the abdomen has been closed and the oral ileal stump brought out through the circular excision.*

If conservative treatment does not relieve the symptoms in the course of a few days, operative treatment must be considered, and this proves necessary in approximately 60 per cent of the cases of ileostomy dysfunction (Warren et al. 1951). When changes are present in the stump of the ileum outside the skin, radial incisions down to the skin through the seromuscular layer at three or four points on the outside of the intestine are recommended. In cases in which the cause is a stenosing process in the skin, circular excision of this may suffice while, when deeper changes are present, establishment of a new ileostomy at another site on the abdominal wall may be necessary.

The author has used a certain technique of operation since 1952 (Lindenberg 1954) in order to counteract ileostomy dysfunction. The abdomen is opened through a lower median laparotomy incision and a circular excision of skin, subcutaneous tissue, fasciae and peritoneum is undertaken at a point in the right iliac fossa marked in advance for the situation of the ileostomy. The fibres of the rectus are divided vertically and the tunnel should admit two fingers and be free from tense margins. The distal loop of the ileum is divided in an unaffected area and both ends are inverted by means of purse-string silk sutures. After division of the mesentery, the proximal end of the ileum is brought out through the tunnel-formed opening to about 3-4 cm above the level of the skin (Fig. 1), in such a manner that the mesentery and the ileum while they traverse the abdominal wall continue their natural intra-abdominal course. No fixation of the intestine and the mesentery to the abdominal wall is undertaken. The stump is screened from the



Fig. 2.

*The purse-string suture has been removed from the ileum and a Paul-Mixter tube inserted, fastened with a double silk ligature round the tube after covering of the intestine and the abdominal wall excision with sterile towels.*

abdominal wall, the purse-string suture is removed and a Paul-Mixter tube with a right-angle bend and triple collar is introduced into the extracutaneous segment of the intestine only (Fig. 2). The intestine is attached to the tube by means of a strong double silk ligature and is lifted by means of the glass tube until the mesentery is stretched but not under tension. A dressing of fluffed-out gauze swabs, each of which only partially surrounds the circumference of the intestine,



Fig. 3.

*Pyramidal dressing with partially circular fluffed-out gauze swabs placed up to the edge of the stump. The dressing supplemented by dressing tissue and cotton.*

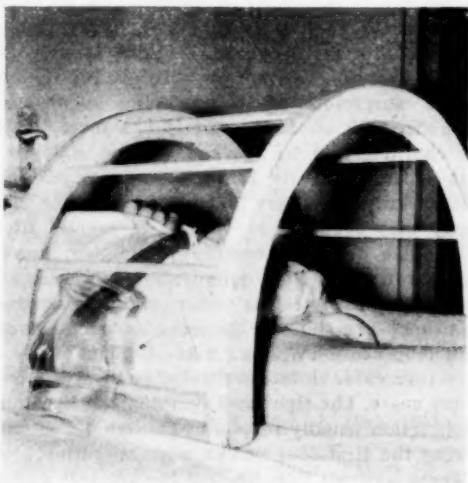


Fig. 4.

*A cradle placed over the patient.*

in then applied. In this manner, a pyramidal dressing which exerts slight compression upon the intestine is formed (Fig. 3). The dressing is supplemented by dressing tissue and cotton and a covering towel so that the tube rests firmly in the original position with drainage to the side. A cradle is then placed over the patient (Fig. 4) and free drainage from the ileostomy is ensured via a rubber tube to a bottle. The patient should be extubated while the dressing is applied to avoid prolapse of coils of intestine during coughing. The intestine should be inspected without removal of the innermost swabs on the second



Fig. 5.

*Appearance of the ileum on the fourth post-operative day, before amputation. Only slight oedema, but fibrin patchings on the external aspect.*



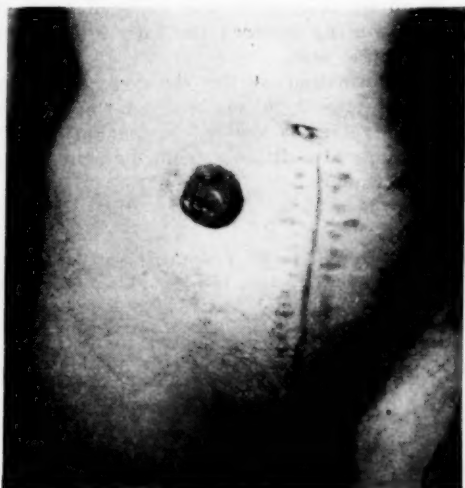


Fig. 6.

Appearance of the ileostomy on the 17th post-operative day, immediately before discharge from hospital. The ileum has healed up, and there is no infection.

day after operation and on the fourth to sixth day after operation the dressing may be removed (Fig. 5). The intestine is amputated to  $\frac{3}{4}$  cm over the level of the skin. The mucosa is sewn to the serosa with atraumatic interrupted chromic catgut and the patient is fitted with a Koenig-Rutzen bag. On the sixth or eighth day after operation the lumen of the ileostomy should freely admit the passage of the index finger and will thereafter shrink somewhat so that after the elapse of three weeks the extra-abdominal part will admit the passage of the fifth finger. The patient must control the calibre of the stoma after discharge. The lumen of the segment of the intestine passing through the abdominal wall will, on the other hand, remain practically unchanged in calibre. Employing this technique the intestine should heal with no reaction in the course of a few days (Fig. 6).

#### MATERIAL

The influence of the operative technique described here upon the incidence of post-operative ileostomy dysfunction will be illustrated by presentation of the following two materials:

**Material I:** This material comprises 55 patients all of whom were submitted to ileostomy on account of ulcerative colitis. The ileostomy was established in the operation wound. Ten of these patients died post-operatively and two of these died in connection with prolonged ileostomy dysfunction, one with multiple perforations of the ileum.

**Material II:** This material comprises 33 patients submitted to ileostomy on account of ulcerative colitis (28 patients), multiple polyposis of the colon (four patients) and cancer of the colon

(one patient). The patients in this material were operated upon according to the technique described by the author with excision of the abdominal wall. Four of the patients died post-operatively from causes not associated with ileostomy dysfunction.

In both of the materials, the principles of pre- and post-operative treatment were the same. In isolated cases only in Material II no primary opening was established from the ileostomy until after the elapse of 24 hours. This factor did not appear, however, to occasion any difference among the patients in this material. In addition, routine amputation of the intestinal stump immediately above the level of the skin was not employed in Material I.

The incidence of post-operative ileostomy dysfunction appears from Fig. 7 which shows that 62 per cent of the cases in Material I developed this complication. In the majority of cases, this complication developed during the second and third weeks after operation and recovery took place in the majority by the 28th day. The symptom which occurred most frequently was colicky abdominal pain. Ileostomy diarrhoea with pronounced loss of fluid occurred in 19 patients. The remaining symptoms were distension of the abdomen, absence of discharge from the ileostomy and vomiting.

No cases of post-operative ileostomy dysfunction occurred in Material II. The volume of the fasting excretion from the ileostomy was approximately 500 ml per 24 hours. In this material, it is a striking observation that in the individual

Days after operation.

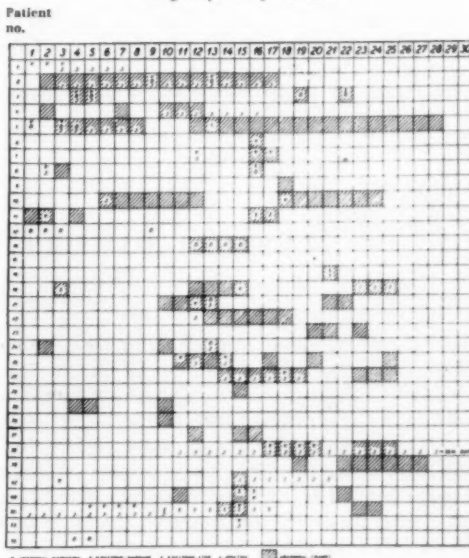


Fig. 7.

Ileostomy dysfunction.

Fifty-five patients operated on by ileostomy.  
33 patients with ileostomy dysfunction.

cases, particularly in very obese patients, in whom a pouch had developed between the ileostomy and the subcutaneous layer on a part of the circumference of the intestine, but with free drainage to the surface, no inflammation with either abscess formation or dysfunction occurred. The reason for this particular condition is perhaps that the tissues of the abdominal wall were excised and that no suturing was undertaken, so that a minimum of necrotic tissue and foreign bodies was present in the region which is exposed to the potent digestive and therefore perhaps infection inhibiting action of the juices of the small intestine.

#### CONCLUSION AND SUMMARY

The complications of the method of ileostomy described are illustrated by review of a material of 33 patients in whom post-operative ileostomy dysfunction did not occur, and this is compared with a material of 55 patients in whom the ileostomy was established in the operation wound and in whom this complication occurred in 62 per cent of the cases. The materials are more or less uniform in other respects and it may, therefore, be presumed that the technique of operation employed in the first-mentioned material was an important reason for the reduction of the incidence of complications. The following advantages are obtained by employing the technique described:

- 1) The fact that the more rigid elements of the abdominal wall are excised implies that compression of the section of the intestine traversing the abdominal wall is reduced or avoided.
- 2) Shrinkage of the extra-cutaneous section of the intestine is avoided by amputation of the intestine close to the skin.
- 3) The risk of infection in the region of the

ileostomy is considerably reduced by omitting suturing between the intestine and the abdominal wall.

- 4) Scar formation at the ileostomy with the tendency to shrinkage and deformity of the skin involved is avoided by establishing the ileostomy at a distance from the actual operation wound.

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## VESICAL DIVERTICULA IN CHILDHOOD

By TYGE CL. GERTZ

Vesical diverticula do not belong to the common urologic anomalies in childhood, although they probably are seen more often than a perusal of the literature indicates. Three of the latest larger pediatric surgical textbooks do not mention them at all (Gross, Grob, Swenson), whereas in special pediatric urology texts they have been given some attention (Campbell, Laurent, Innes Williams).

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The fact that they are so unheeded is probably not only because they are so uncommon but also because the symptoms are comparatively uncharacteristic, and can be difficult to recognize despite modern roentgenologic methods, particularly micturating cystogram and cystoscopy.

The latest major publication about bladder diverticula in childhood is Kretschmer's from 1934. He collected 19 cases from the literature, the earliest one dated 1859, and added 6 of his own. Campbell reports having operated on 18, giving no further details, Laurent has only seen 3 while Innes Williams gives

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Fig. 1.



Fig. 3.

neither information of personal cases nor of the frequency. It so happens that we have recently treated two children with vesical diverticula. Based upon these two cases, together with two earlier cases, a comment on the etiology, symptomatology and therapy of this anomaly is presented.

*Case No. 1.*

Three-year-old boy admitted from local hospital for suspected bladder diverticulum. Acutely ill two months previous to admission with febrile urinary infection, resistant to treatment. No incidents of retention or micturating disturbances. Micturating cystogram reveals a large right-sided diverticulum (Fig. 1), vesico-ureteral reflux and ureterocele on the left side. Cystoscopy shows normal bladder with no trabeculae and verifies the big diverticulum into which the right ureter opens. Signs of bladder neck obstruction or

urethral valve were not found. On operation the diverticulum is excised and the right ureter re-implanted into the bladder. On discharge, three weeks after operation, micturition is free and the urine sterile.

*Case No. 2.*

Eight-year-old boy with acute attacks of retention since four years of age. No verified pyuria and between attacks the micturition is free. Micturating cystogram shows abnormal, seemingly bipartite bladder, insufficient voiding but no reflux (Fig. 2). In the vicinity of the left ureter, which is undetectable, cystoscopy shows a wide cavity into which the cystoscope can be inserted, thus disclosing a large diverticulum of nearly the same size as the bladder itself.



Fig. 2.

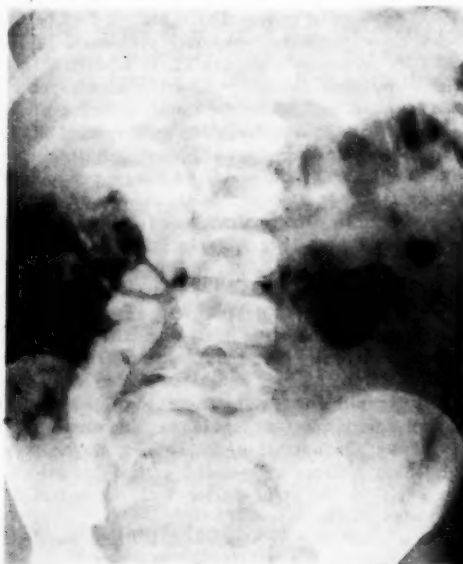


Fig. 4.

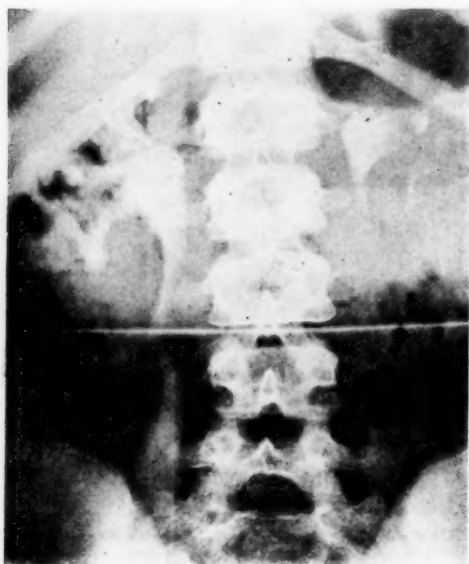


Fig. 5.

On the right side a large ureteric orifice is revealed, allowing the ureter catheter to be introduced 3—4 cm. On operation a giant diverticulum is removed, into which the left ureter opens, together with a small right-sided diverticulum erroneously supposed at cystoscopy to represent the right ureteric orifice, but independent of the right ureteric orifice. Re-implantation of left ureter is necessary. Uneventful convalescence. On discharge micturition is free, urine sterile and no reflux is revealed on cystography.

#### Case No. 3.

4½-year-old boy who since the age of 18 months has suffered from recurrent attacks of acute retention. No verified pyuria and symptom-free between attacks. An explorative cystotomy performed in local hospital provides no explanation. Is admitted to the Pediatric Surgical Service, where the attacks at first are interpreted as bladder neck obstruction, especially in regard to the cystotomy findings. Repeated bladder neck resections are performed, proving quite ineffective on the considerable residuum. Roentgenologically, the correct diagnosis is hinted at, as cystography suggests a large diverticulum, sited behind the right side of the bladder. (Fig. 3). Dilatation of right ureter and pelvis is demonstrated, but no reflux (Fig. 4). Cystoscopy reveals normal bladder mucosa with no trabeculae. Orifice of diverticulum is not seen.

On readmission eight months later there have been no attacks since discharge, but considerable residuum persists. Cystoscopy now revealed both ureter orifices markedly gaping, the right more than the left, but still no positive reflux could be demonstrated on cystography. On operation the correct diagnosis was established: A large right-sided vesical diverticulum. After excision the right ureter was re-implanted in the fundus of the bladder.

On discharge voiding was normal, urine sterile, but now a vesico-ureteral reflux to the left ureter was demonstrated. Re-examination five years later showed

the boy in good health. Ambulant urogram was practically normal, in particular the dilatation of both the right ureter and pelvis has disappeared in spite of the fact that during the operation the right ureter was implanted direct into the bladder. Furthermore it was noticeable that there was no dilatation on the left side, despite the reflux demonstrated five years earlier. (Fig. 5).

#### Case No. 4.

Twenty-month-old boy with recurrent pyuria since the age of ten months. Multiple anomalies are demonstrated, namely hydronephrosis, hydroureters, hypertrophied trabecular bladder and a large left-sided bladder diverticulum independent of left ureter. During admission, which lasted nearly twelve months, the patient was treated with diverticulectomy plus cystostomy and — later on — ureteral resection and nephrostomy. Is discharged in tolerable health though sub-uraemic and with pyuria. Dies the day after second admission two years later. Possible glomerulonephritic complications revealed at autopsy.

#### ETIOLOGY

It is well established that vesical diverticula in adult age are caused by an infravesicular obstruction or hindrance in the sphincter apparatus of the bladder. Contrary to this, the etiology of diverticula appearing in early childhood is much more questionable. In 1940 Kretschmer published a material of 236 vesical diverticula in all age groups, of which only nine were less than ten years of age. He stressed the point that also in childhood there is always a previous obstruction and presented the following percentage distribution of causes: prostatic obstruction 67 per cent, "median bar" 15 per cent, bladder neck obstruction 8 per cent, prostatic cancer 7 per cent, urethral stricture 2 per cent, congenital urethral valve 1 per cent. In connection with this it may be observed that in 1934, as already mentioned, he described 25 cases of vesical diverticula in childhood, of which 19 were derived from the literature. He stated the following origins: phimosis two cases, congenital valve three cases, bladder neck stenosis two cases: In four cases no obstruction was found, in 14 cases the pathogenesis remained unexplained. Three of the four cases with no signs of obstruction were published by Hyman in 1923, all cured by simple excision of the diverticulum.

Badenoch (1949) has recorded 26 cases of bladder neck stenosis mainly diagnosed in adults, although the youngest was only seven years old. All cases were complicated either by diverticula (23) or hydroureter. He considered bladder neck obstruction the most important source of vesical diverticula, and states that he has seen only two cases without this, just as he stresses never having seen diverticula as a complication of prostatic hyperplasia. Trabeculated bladder was present in all his cases and nearly all the diverticula were sited in the region of the ureteric orifice.



Regarding bladder neck stenosis to be a congenital disease, as *Badenoch* does, the vesical diverticulum must be considered a rather late secondary complication, chiefly observed in adult age. In our material of bladder neck stenosis in childhood, we have at any rate been unable to demonstrate any case of diverticula.

*Ashton Miller* (1958) has published a material of 105 cases of vesical diverticula, of which five were under the age of 15. In all cases he found hypertrophy of the bladder wall. The majority of the cases were due to bladder neck stenosis, and it is his opinion that diverticula are always caused by a hindrance to the outflow.

*Campbell* stresses the point that the diagnosis congenital vesical diverticulum can be acknowledged only in cases where obstruction can be excluded with certainty, and thus defined it is a rare anomaly. He demonstrated the anomaly in three autopsies, infants from seven days to three months of age. As mentioned above he has operated on 18 cases, on which he only offers the information that diverticula in the majority of cases probably were secondary to an obstruction.

Also *Innes Williams* emphasizes that vesical diverticula in children are mainly found coupled with obstructions, although he admits having seen cases where the bladder was quite normal without obstruction. *Innes Williams* refers to various older theories of the genesis, such as that of *Englisch* from 1880. (*Laurent* has also mentioned this, oddly enough as "la theorie anglaise"). *Englisch*, who was German, believed that the origin of the vesical diverticula could be a temporary closure of the urethra during foetal life. This, however, seems hypothetical. Children born with congenital urethral obliterations have excessively dilated upper urinary tracts and bladder, but no diverticula. *Caulk's* theory (1929) seems more convincing. He assumes that the congenital diverticulum has developed from an additional blind ureter. *Innes Williams* has at any rate observed a specimen where this theory fits in excellently, probably one of the cases of which he gives illustrations and which seems identical to our three first cases.

These three cases may undoubtedly be regarded as genuine congenital diverticula. In all three cases the bladder wall was perfectly normal without hypertrophy or trabeculae. Neither cystoscopy, cystography nor operation revealed signs of obstruction or bladder neck stenosis. The cases may not provide any definite explanation as to the genesis of the congenital diverticula of the bladder, but, as already mentioned, *Caulk's* hypothesis seems most suitable, if one compares the identical siting and the intimate relationship with the normal ureter. At any rate this is valid in our two cases from 1958 and probably also in the third, even if it was taken for a bladder neck stenosis before the operation proved it to

be a diverticulum. The fact that the normal ureter was found opening into the wall of the largest excised diverticulum might simply be the result of the orifice being drawn out into it during the growth of the diverticulum.

Our fourth case is much more difficult to explain retrospectively, but presumably it was caused by some congenital voiding hindrance; cf. the trabeculized bladder and distension of ureters and pelves. The fibrous posterior urethra demonstrated at autopsy also suggests this. It might possibly be compared with what *Bodian* describes as fibroelastosis urethrae. Autopsy gave no exact explanation as to the nature of the disease, but it seems reasonable to believe that in this case the diverticulum belonged to the more frequent secondary type.

#### SYMPTOMATOLOGY AND DIAGNOSIS

There is no pathognomonic symptom of vesical diverticula. In cases where the diverticulum is considered secondary the basic disease will dominate the symptomatology. "Miction en deux" is considered a classic symptom, but in childhood this is probably more often seen in connection with megaureters with reflux. In cases with recurrent urinary infection the probability of vesical diverticulum should also be recalled, although various other anomalies in the urinary tract are capable of provoking these attacks. According to *Innes Williams*, acute retention, as observed in our two cases, is often caused by vesical diverticula. Probably this can be explained as a mere mechanical phenomenon. If the bladder is not emptied duly on demand the diverticulum will distend considerably and may displace or actually block up the posterior urethra, thus even complicating catheterisation. (See case report 2.)

Sometimes the diagnosis can be made solely on palpation of the abdomen, where the diverticulum is felt as a rather asymmetric swelling above the symphysis, not decreasing after micturition. In the vast majority of cases, however, it is necessary to use more exact methods of examination, such as cystography and cystoscopy. Cystography is an excellent method, which also may provide information as to possible reflux to the ureters and also as to the condition of the sphincter and urethra, though it should be realized that a frontal roentgenogram is quite inadequate. Even large diverticula can be masked by the shadow of the bladder, although they may be revealed after voiding if retention is present. Oblique or lateral exposures are usually demanded. *Innes Williams* recommends the projection used in pelvimetry. The most important examination, however, is cystoscopy, though confusion with a gaping ureteric orifice is possible, as diverticula most frequently originate from the vicinity of the orifice and the latter is not infrequently found inside the diverticulum. If the diverticulum is large enough the cystoscope can usually be intro-

duced and the ureter orifice eventually detected. Only in our two first-mentioned cases was it possible to make an exact diagnosis preoperatively; in one of them a right-sided diverticulum was unexpectedly found, its opening being confused with the ureter orifice. Such mistakes should be avoidable when cystoscopy is compared with the cystography findings. Lack of uretero-vesical reflux seems to indicate that what was seen must be the opening of the diverticulum.

#### THERAPY

When diagnosis is established, operative treatment of a vesical diverticulum nearly always is indicated, even if there are no current symptoms. Correction of a potential basic bladder neck stenosis or other obstruction is obviously necessary. We have found the transperitoneal approach to these anomalies in childhood most satisfactory. The exposure is excellent and it is surprisingly easy to dissect these very deep-seated diverticula all the way down to the junction with the bladder. A thing which has caused some trouble is the normal ureter, this being quite adherent to the diverticulum wall. In the cases where the ureter has opened into the diverticulum it has been necessary to resect as close to the orifice as possible. During recent operations we have re-implanted in the resection-line and sought to give the ureter a submucous course in order to prevent reflux.

## CYSTOMETRY

### A NEW TECHNIQUE APPLYING A PERCUTANEOUSLY INSERTED CATHETER IN THE BLADDER

By E. SANDØE, J. BRYNDORF, and TYGE CL. GERTZ

Cystometry, the measurement of intravesical pressure at various fillings of the bladder and during micturition, appears to have been effected exclusively by means of a catheter inserted through the urethra. Performed in this way the examination will give information about the rigidity and contractility of the bladder-wall, but will tell us nothing about the conditions of passage in the ostium of the bladder and in the urethra, primarily because the placement of a catheter inside these structures will alter the lumen, secondarily because catheterization in men and

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**CONCLUSION**

Vesical diverticula in childhood is certainly no common disease, although it is an anomaly which must be recalled in cases of recurrent urinary infections and especially retention. Vesical diverticula may appear as isolated, congenital abnormalities without signs of obstruction in the lower urinary tract, but according to the literature they are more often secondary to a bladder neck obstruction. 3 out of the 4 cases presented here must be considered as primary, genuine congenital diverticula, while the fourth probably was secondary, though the fundamental disease was never verified. Treatment should in all cases be operative. The transperitoneal approach is recommended, presenting no complications. Bladder neck stenosis must either be excluded or treated in connection with the diverticulectomy.

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boys will quite frequently cause dysuria and sphincteral spasms of long duration. In order to overcome these drawbacks we have worked out a new technique of pressure measurement using a polyethylene catheter inserted percutaneously into the bladder.

The procedure was inspired by a modified Seldinger technique which was designed for the insertion of polyethylene catheters into peripheral arteries and has been in use for five years in the Cardiological and Radiological Departments of Rigshospitalet (Thomsen & Tybjærg Hansen 1954).

#### METHOD

For the puncture is used a cannula, outside diameter 1.6 mm, with an obturator; a nylon conductor, 30 cm long and 0.8 mm thick; and a



Figure 1.

Schematic drawing of the catheter with the nylon conductor inserted.

1. The conically formed point of the catheter.
2. The sidehole of the catheter.
3. The plastic tubing which is to be used as a stop.
4. Metal screwcap.
5. Nylon conductor.

polyethylene catheter (Fig. 1). The catheter is 20 cm long, its circumference equal to that of the cannula, and its inside diameter 1.0 mm, so that it can just slide on over the nylon conductor. At one end the catheter is tapered and at a distance of two cm from the conical point equipped with a small sidehole for the pressure measurement. At the other end the catheter is furnished with a small collar and a screw-cap with internal thread, so that the catheter can be connected through a nylon catheter to the manometer and the water container. Before being used the catheter is pierced perpendicularly through the middle of a small piece of plastic tubing, which is used as a stop in order to ensure that the pointed end of the catheter — once it is inside the bladder — stays intravesically until the examination is finished. For pressure measurements has been used a Tybjaerg Hansen capacity-manometer; the pressure curves have been registered by a photographic recorder.

#### CONSTRUCTION OF CATHETER

The tapering of the catheter is obtained by stretching the rather tough and plastic catheter over the nylon conductor as a mandrel and then cutting off the catheter at right angles to the axis at the spot where the wall has been drawn thin (Thomsen & Tybjaerg Hansen). The collar of the catheter is made by heating the other end until the polyethylene is at the point of melting whereupon it is quickly, but very gently, pressed against a cold, plane surface. The side-hole is punched with a drift. The catheter is sterilized by immersion into a 0.1 per cent benzalkon solution for twenty-four hours.

#### TECHNIQUE

At the beginning of the examination the bladder should be well filled and traceable by percussion three to five cm above the symphysis. This state is generally obtained by making the patient drink copiously during four hours prior to the examination and without micturition. If the bladder is not filled up to the level required,

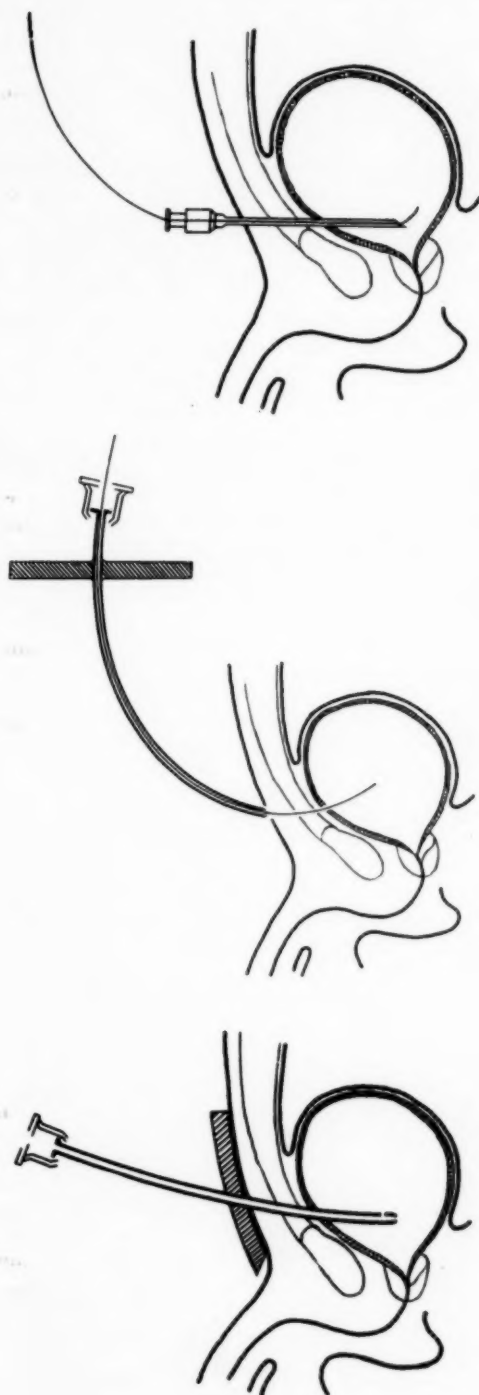


Figure 2. (a, b, &amp; c).

Schematic drawings showing the various stages during insertion of the catheter. Both cannula and catheter are highly over-dimensioned as compared to the size of the bladder.

the examination must be postponed, and the patient requested to go on drinking. In girls the bladder may be filled up through a urethral catheter, a procedure that should not, however, be followed in boys or men because of the risk of sphincter spasms and dysuria.

Before puncture of the bladder a local anesthesia is applied cutaneously or subcutaneously immediately above the symphysis. A small incision is made through the skin near the midline at the upper edge of the symphysis and the cannula is inserted with direction perpendicularly towards the abdominal wall. After puncture of the bladder the nylon conductor is passed through the cannula (Fig. 2 a), and the latter is removed. Next, the polyethylene catheter is slid on over the nylon conductor until the latter projects from its distal end (Fig. 2 b), and, finally, the polyethylene catheter and the nylon conductor are pushed jointly through the walls of the abdomen and the bladder. When 4 or 5 cm of the catheter is in the bladder, the nylon conductor is removed (Fig. 2 c) and the catheter is fixed to the abdominal skin with tape, which is applied across the plastic tubing. The catheter is then connected up with the manometer and the water container. This technique will cause the bladder-wall to close tightly round the polyethylene catheter. At the end of the examination the bladder is emptied by aspiration through the catheter before the latter is removed.

#### RESULTS

Until now we have carried out 10 cystometries employing the technique here described. The patients were children aged from 1½ to 14 years, four boys and six girls. Seven patients had two catheters inserted in preparation for continuous

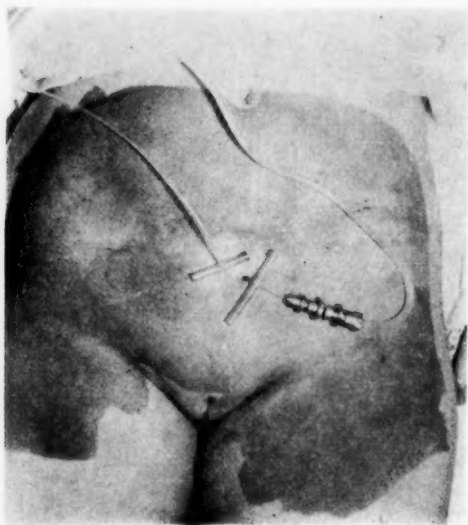


Figure 3.

Patient with two polyethylene catheters percutaneously inserted into the bladder.

pressure measurements during slow filling of the bladder, and three patients had one catheter inserted. In seven cases the urine was sterile, in three there was pyuria. The examinations all passed off smoothly without complications of any kind. After insertion of the catheter the patients appeared to be feeling no discomfort and could be induced to urinate in the sitting or standing position, the micturition being painless. In most cases the pressure measurements through the catheter went on for nearly two hours, and immediately after having had their catheters removed every one of the patients could join in the play of the other children in the ward. Two patients underwent cystoscopy a few days later, one (a case of pyuria) underwent cystotomy, and no case showed any change that could be ascribed to the puncture.

#### DISCUSSION

The advantages of the new technique are: The percutaneous catheter does not interfere directly with conditions of passage in the bladder neck or the urethra, and is, moreover, so thin and flexible that it will hardly irritate the mucosa of the musculature in the bladder-wall to the extent of upsetting the tonus of detrusor or sphincter. Furthermore, insertion of the catheter, carried out in local anesthesia, involves but very slight pain, and the patient — once the catheter is in place — feels no discomfort — in fact, it does happen that he forgets all about it.

The percutaneous bladder catheterization, therefore, makes it possible to do cystometry under more physiological conditions than hitherto, and to obtain a reliable determination of the pressure in the bladder during micturition. This, consequently, opens up the possibility of determining a factor ( $k$ ) which characterizes the passage conditions in the neck of the bladder and in the urethra, and can be used as a measure of the degree of any existing stricture (Bryndorf & Sandøe 1959). The factor  $k$  can be computed

from the formula  $k = \sqrt[3]{\frac{2g}{p} \frac{v}{v}}$ , in which  $g$  re-

presents the acceleration due to gravity,  $p$  the intravesical pressure, and  $v$  the corpuscular velocity in the jet of urine, which can be determined from the size of the parabola described by the urinary jet. The formula is derived from the physical laws applying to turbulent flow under stationary conditions in pipes with smooth walls. The factor  $k$  is an abstract number varying from 1 to infinity. In patients with a normal urethra it is likely to be in the vicinity of 1 whereas it will be higher in patients with stricture. The rise in  $k$  will be proportionate to the hydrodynamic significance of the stricture. We have so far carried out the determination of  $k$  in two patients. In one, a boy with bilateral hydronephrosis, it was found to be 2.3. In the other, a girl not



offering any clinical symptoms of hindered outflow of urine from the bladder,  $k$  was determined to 1.2.

#### RISKS

The percutaneous bladder catheterization begins, as already mentioned, with a suprapubic puncture of the bladder, a surgical measure that has been employed from of old for treatment of acute retention of urine. On this subject Dahl-Iversen says in his textbook on Operative Surgery of 1950: "Punctio vesicae will be considered in urinary retention in cases that do not lend themselves to catheterization. It may be repeated as often and for as long time as deemed necessary". Statements by Boeminghaus, Boshamer, and Wehner are on line with this although, at the same time, advising the use of a thin puncture cannula in order to avoid extravasation of urine into cavum Retzii with consequent risk of infection.

Guttmann and Kretschmer state that the procedure involves little risk of peritonitis in consequence of perforation of peritoneum and intestinal lesions. Campbell (1954) says that the suprapubic puncture "involves some risk in patients of all ages because of the danger of inciting deep bleeding or the occurrence of hematoma, perivesical urinary extravasation or phlegmon, and especially as it may involve the space of Retzii". However, in the same work he tells us that he has performed catheter insertion with a special trochar number 21 F "in several infants and children even in the first week of life, and have seen no untoward complications"; also that he considers this form for bladder catheterization sufficiently safe to justify its application to outpatients. Our own experiences covering 17 punctures in 10 children were, as already mentioned, favourable in that we have seen no complications. It should be emphasized, however, that the punctures were done exclusively in patients whose bladder could be traced by percussion not less than two fingerbreadths above the symphysis.

On one not unimportant point the risk attending percutaneous bladder catheterization seems

to be less than that involved in catheterization through the urethra: it should be possible to carry out the former under conditions of perfect sterility and thus obviate all dangers of infection in the urinary system.

In cases where a mediana laparotomia has been performed a certain caution as regards the examination would be advisable. In such patients adhesions will often be found, binding peritoneum down to the symphysis, and the prevesical peritoneal fold will not infrequently contain adherent intestines which would mean an added risk of intestinal lesion.

#### SUMMARY

A description is given of a method for percutaneous bladder catheterization that is quick, that seems to involve but small risks, and that presents very little subjective discomfort to the patient. It opens possibilities for determination of the pressure in the bladder during micturition, which is of diagnostic importance in cases of suspected stenosis in the bladder-neck or the urethra.

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## EFFECT OF HUMAN SERUM ON GROWTH OF PLANT ROOTS

By FOLKE RØNNIKE

## INTRODUCTION

Oligodynamic substances, *i. e.* substances characterised by the vigorous physiological effect produced by very small quantities of them, play an extraordinarily important part in the biological interaction between the vegetable and animal kingdoms. Naturally, interest has primarily been focused on the responses of the animal organism to substances originating from the vegetable kingdom. In this respect the vitamins hold a prominent position, while the knowledge of the responses produced in plant by oligodynamic substances from the animal organism is still very scanty.

In the 1930's some interest was, however, aroused in the effect of animal hormones on higher plants. The first investigations in this field were carried out by Schoeller & Goebel (1931); in Denmark by Gad-Andresen & Jarløv (1934). The latter authors availed themselves of the same experimental technique as Schoeller & Goebel and arrived at the same conclusion, *viz.*, that in all probability the promoting effect of sex-hormone preparations on the germination and growth of hyacinths was not due to the sex hormones proper but to impurities in the preparations. The hormone preparations used were produced from urine, which is known to contain large quantities of plant growth substances which possess no sex-hormonal character.

The results of the experiments on the effect of animal hormones on higher plants were summarized in a paper by Clara Zollikofer (1938). She mentions that the pure, crystalline oestrone is known with certainty to have a nonspecific, growth-promoting effect, and that all other effects of oestrone observed in plants could secondarily be ascribed to this fact. The quantities of oestrone necessary to produce any effect at all were, however, greatly in excess of the quantities which were effective in animal experiments (1,000 to 10,000 times). It was, therefore, hardly justifiable to talk of any proper hormonal effect on plants.

In addition to oestrone, other animal hormones were able to produce a similar nonspecific, growth-promoting effect.

In 1940 and 1945 Askill & Doris Löve published accounts of experiments on the effect of animal hormones on dioecious plants (*Melandrium dioicum*). In addition to the above-mentioned growth-promoting effect in general, the animal hormones were found to produce an effect on the sexual character of these

plants. When treated with oestrone paste, *Melandrium* displayed a distinct female predominance, while plants treated with testosterone displayed a distinct male predominance.

Helmkamp & Bonner (1953) investigated the influence of 17 different steroids on the growth of aseptic pea cultures. Only oestrone had a definitely promoting effect on the growth of stems, while testosterone presumably had an inhibitory effect.

Also substances from placenta, the large hormone-producing gland, have been used in experiments on the growth of plants. Mönckeberg (1947), in fertilising plants with a placenta extract, found a very vigorous growth promotion in leaves and stems as well as in flowers. He states cautiously, however, that it is impossible to draw any conclusion therefrom as to whether the effects found originate from definite animal hormones, but that the question may be one of a nonspecific fertilising effect.

Fertilising of plants with manure from domestic animals is presumably as old as agriculture itself, but the scientific research on the plant-growth-active substances in solid and liquid manure has up to recent years mostly been concerned with the inorganic components of these substances. The discovery of plants hormones has, however, induced scientists to take a greater interest in the organic constituents of manure.

Incidentally, some of the first experiments on the content in urine of organic plant-growth substances were made on human urine and not on cattle urine.

Beyond what is mentioned above, information about the effect of oligodynamic substances of animal origin on higher plants is found only sporadically in the literature and is, therefore, not immediately accessible.

It must be considered beyond doubt that the vital processes in higher plants are contingent on hormonal regulation. Compared with the number of animal hormones, few genuine phytohormones are known; in fact, perhaps, a single one only, *viz.*, 3-indolacetic acid (3-IAA). Substances influencing the cell elongation are termed auxins, and the most important of these is 3-IAA, which is supposed to originate from tryptophane via a number of intermediaries, ending in indolacetaldehyde as the immediately preceding stage (P. Larsen, 1944). Other plant hormones, though so far only hypothetic, are such as may be assumed to determine the transition of the plants from the vegetative stage to the generative one.

Besides naturally occurring auxins, a great number of organic, synthetic auxins are known, comprising, *inter alia*, certain indole and phenoxide derivatives, such as 2-4-dichlorophenoxyacetic acid, parachloro-

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phenoxybutyric acid, various derivatives of naphthalene acetic acid, naphthoxyacetic acid, as well as certain benzoic-acid derivatives, *i. e.* relatively simple organic compounds. Between 1942 and 1945 a group of British plant researchers, Slade, Templeman & Sexton (1945), and others, were the first to discover these synthetic, active substances, and in a certain respect plant-hormone research may thus be said to be a comparatively young branch of science, a mere 15–20 years old.

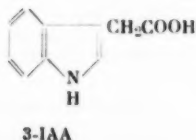
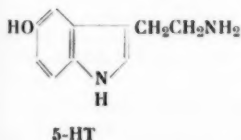
So far, nothing is known about the difference in the function of the genuine, naturally occurring auxins and the synthetic, "hormone-like" herbicides beyond that, in order to become active, the latter must have far higher concentrations than those occurring in nature.

Common to all the synthetic substances is, however, that but a slight change in their chemical structure or stereo-isomerism may completely change their properties, and from this fact important details about the chemical structure of the auxins as well as of their mode of action have been derived. The mode of action of the auxins seems to be contingent, among other things, on the presence in them of a carboxyl group and some atom group in a relative ortho-position, capable of reacting with peptides and -SH-groups, respectively.

Incidentally, it should be mentioned that the effective auxin response in the living plant tissue is assumed to be a resultant, not only of the tissue's own auxin concentration but also of the concentration of a number of naturally occurring auxin antagonists, some of which are assumed to be derivatives of coumarin, which is also zoophysiologically active.

It is beyond any doubt that, especially in high concentrations, auxins are mitosis-producing under extremely varying conditions (irregular division of proliferating tissue and formation of rootsuckers in pericambia, etc. may be mentioned (Burström (1953)). The study of the effect of auxins is thus an immensely fascinating field with a general biological aim in view and, consequently, also a bearing upon human medicine.

For the past 7 or 8 years researchers within human- and zoo-physiology have been working intensively with the substance 5-hydroxytryptamine (5-HT), the structural arrangement of which resembles that of 3-IAA very much:



5-HT may be the counterpart in the animal organism of the 3-IAA in plants. Wolley (1957) points out that the two substances are closely related to each other, 3-IAA being merely the acid found in the acid medium of the plants, while 5-HT is a hydroxylated, closely related base found

in the basic medium of the zoo-organism in higher vertebrates.

In the carcinoid diseases the segregation in the urine of 5-hydroxyindolylacetic acid, which is assumed to be a segregation product of 5-HT, is greatly increased (Page et al., 1955).

If 5-HT is the counterpart in the animal organism of 3-IAA in plants, a similarity in the physiological effects of these two substances may be found. Although these effects are not known with certainty for both substances, there is, however, something which indicates that they are both important for the permeability conditions of cells (Pickles & Sutcliffe, 1955).

Wolley further points out that the lack of similarity in the structures of substances with auxin activity in plants corresponds to what is known within the animal kingdom in respect of substances with a 5-HT-like effect. Incidentally, similar conditions are known to exist elsewhere in the animal kingdom; substances, for example, with structures deviating greatly from that of oestron may have the physiological effects characteristic of this hormone (*cf.* also vitamin K, etc.).

Only scant information about investigations on the occurrence of plant-hormone-like substances in the animal kingdom is available, and they are, indeed, difficult to demonstrate. The biological measuring methods employed, such as the *Avena* coleoptile curvature test, the slit pea stem curvature test, the *Avena* section test and root-growth tests, often in conjunction with paper chromatography, are not so readily accessible.

The *Avena* coleoptile curvature test was employed by Kögl and co-workers in Holland from 1933 and onwards. These researchers investigated the auxin activity of urine under varying conditions, and found no dependence on age and sex, no relationship to pregnancy or menstruation, nor any increased segregation of auxin in the case of cancer, tuberculosis or diabetes. On the other hand, the segregation after meals was increased, especially following the consumption of salad oil. The average segregation amounted to about 2 mg auxin per 24 hours, but in one case (an 18-year old man, in whom there was otherwise nothing abnormal) the segregation was found to be greatly increased — up to 10 mg. Kögl and co-workers concluded that the human organism itself must be capable of producing auxins. They believed it was possible to isolate three different auxins from urine; one of them, being identical with 3-IAA, they named heteroauxin.

Wieland, de Ropp & Avenier (1954) checked Kögl's results by means of the pea stem curvature test, but succeeded in finding 3-IAA only. Bennett-Clark et al. (1952), too, were unable to detect any auxin other than 3-indolylacetic acid in paper chromatograms of an ether extract of urine. The measuring method adopted was elongation of subapical sections of *Avena* coleoptiles. Bentley (1958) emphasises, however, that different extraction solvents and different chromatography solvents give different auxin pictures, both qualitatively and quantitatively.

Since 1908 (Herter), IAA has been known to be a constituent of urine. A staining reaction characteri-

stic of IAA has been known since 1882 (Nencki & Sieber).

In 1953 De Landsheere examined the effect of different auxins on the blood picture in guinea pigs and found a distinct eosinophilia. Kodicek et al. found, under certain conditions, a pellegra-like response in rats fed with 3-IAA. Simonnet (1953) is of the opinion that there is no carcinogenic effect of 3-IAA on the animal organism. In this connection it should be observed that IAA no doubt plays an important part for the neoplasms of plants, the so-called galls, both for the galls proper and for their metastases (Gautheret 1953).

For the investigation of the interaction between the physiology of the animal and vegetable kingdoms in the field of the oligodynamic substances — and primarily for this purpose — a root growth test has been introduced by D. I. Mach t and M. Livingston 1922. With this method, Mach t has published a great number of studies during the last 30 years on the effect of human serum, cerebrospinal fluid, and urine on the growth of *Lupinus albus* roots.

He has demonstrated in a large number of papers that, under certain standard experimental conditions, sera from healthy persons inhibit the growth of lupine roots. With some human diseases the inhibition is, according to his investigations, more pronounced than when sera from healthy persons are used. His first paper on this subject came out in 1922. In 1941 he published a comprehensive statistical assessment of his method. Later on, he has prepared several further relevant papers. The latest one, which appeared in 1955, deals with the specially high toxicity to lupine roots of urine from cancer patients.

In the twenties and thirties several authors tried to reproduce Mach t's results, but the outcome was not unequivocal. U p j o h n et al. (1928), whose account of their control experiments is most detailed, were able to confirm that the inhibition of sera was about 70—80 per cent of the standard value, whereas they were unable to bear out the presence of any further toxicity in sera from patients suffering from the diseases or conditions which, according to Mach t, should bring about such toxicity.

Mach t's continued works, especially that of 1941 and the paper of 1955 with the sensational results, have induced the present author to make still another critical examination of his methods and diagnosing of diseases by means of serum studies.

#### TECHNIQUE

##### *The Lupine Root Test of Mach t-Livingston*

Within plant physiology there are, as mentioned, many quantitative measuring methods for the determination of auxin (Boysen Jensen 1935, Larsen 1955, Linser & Kiermayer 1957). Below, an account will be given of the lupine root test method of Mach t-Livingston

or, in brief, Mach t's method, which, in the way in which it is being carried out today, originates from 1922. The Italians Benedicenti & De Toni (1901) were, however, the first to use lupine roots for quantitative determinations of root growth factors. Fundamentally, the lupine root test is identical to the root growth methods described by Moewus (1949), Burström (1953), Hansen (1954) and Åberg (1954).

Audus (1948) points out that in numerous studies it has been demonstrated that there exist definite relationships between elongation of roots cultivated in different concentrations of growth-promoting solutions (auxins) and these concentrations. Larsen (1955) claims that the sensitivity to auxin is generally higher in young roots than in young stems and coleoptiles, but he further mentions that the root-growth methods are, however, not specific. Reinert (1950) has demonstrated that low concentrations of acetic acid are capable of producing growth inhibitions similar to those obtained with auxin. Clauss (1952) demonstrated a considerable influence, specific or nonspecific, of several organic acids and of  $\text{Na}_2\text{HPO}_4$  on the growth of the roots of cress.

In this work the inhibitory influence of serum

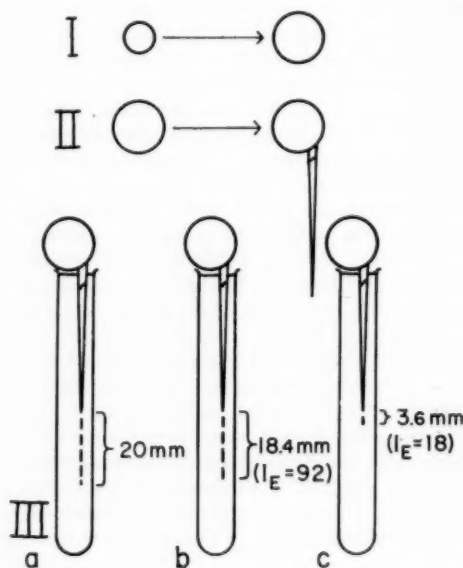


Fig. 1.

Procedure of the lupine root test (shown schematically).

I: Imbibition in water (20°C, 24 hrs.).

II: Preliminary growth in sphagnum (or in filter paper tubes; 20°C, about 60 hrs.).

III: Growth in solutions (15°C, 24 hrs.).

a) Growth in Shive's solution (comparison growth: 100 per cent).

b) Growth in serum solution. Maximum 92 per cent.

c) Growth in serum. Minimum 18 per cent.



solutions on the growth of roots of *Lupinus albus* L. is investigated according to Macht's method.

The principle of the method appears from Fig. 1. Dried seeds, with a diameter of about 9–11 mm, of a bitter *Lupinus albus* variety are soaked for 24 hours at 20° C, after which the imbibition has reached its maximum. They are then placed in a preliminary germination medium, the character of which may differ (damp air in moistened filter-paper tubes placed in special racks; washed "Nodampoff" sphagnum containing 300 per cent water). During the preliminary germination the root appears, the size and appearance of which are as shown in the sketch, Fig. 1. The seedlings are then suitable for use in experiments and are, under certain standard experimental conditions (15° C, 24 hours), transferred to nutrient solutions in test tubes for further growth, *i. e.*, partly to Shive's three-salt solution, partly to the solutions which it is desired to investigate. The lengths of the roots before they are placed in the solutions are termed the *initial lengths*. The increment in Shive's solution, which is shown first, serves as a comparison value, while the increment in the other solutions is expressed as a percentage of the comparison value. The figures arrived at are termed the phytotoxic indices (indices of elongation,  $I_E$ ) of the solutions. As will be seen, serum has a phytotoxicity fluctuating between the empiric maximum, *i. e.* index 92, and the empiric minimum, *i. e.* index 18. Otherwise, the technique will appear from the author's previous works on this subject (Rønnike 1957a, 1957b, 1958). As is the case in all plant-growth experiments, the carrying-out of the technique is a lengthy affair, a single experiment requiring about 20 hours' work and lasting more than five days.

### *Lupinus albus* L.

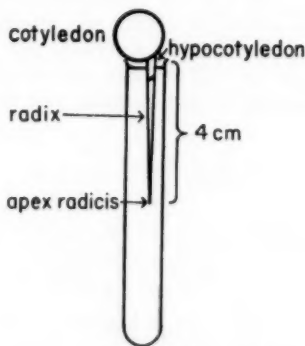


Fig. 2.

Sketch showing factors influencing the growth of the lupine root in nutrient solutions.

**Cotyledon:** Temperature-insensitive within wide limits.

**Apex Radicle:** Centre of the growth control.

The growth of the root takes place by two stages: Near the tip a cell-division takes place, and at a short distance behind the tip the newly formed cells begin to elongate. Apex is highly temperature-sensitive. The growth rate also depends on the temperature and the humidity during the preliminary growth. Further, the growth is dependent on the initial root length, the  $O_2$ - and  $CO_2$ -tension, chemicals contained in the solution, etc.

The mechanisms comprised by the growth of a plant root are, of course, as intricate as those involved in the growth of the animal organism.

In the sketch, Fig. 2, an attempt has been made to give a review of some of the factors which are important to the elongation of lupine roots in nutrient solutions.

A conspicuous difference between the plant organisms and the human organism is found in the fact that the plant organisms are able to grow over a wide temperature range. The intermediary metabolism, and thereby, presumably, their responsiveness, may therefore be assumed to be different at different temperatures. The "centre" of the growth mechanisms is located in the root tip proper and is highly sensitive to the temperature of the nutrient solution. It may, however, also depend on various factors during the period of preliminary growth. The author has demonstrated how, quite particularly, the water content of the preliminary culture medium is important for the later increment of the roots in the nutrient solutions, just as in these solutions the increment is also dependent on the initial length (Rønnike 1958). Furthermore, the temperature during the preliminary growth is important, while, within wide limits, the cotyledon temperature has but slight influence on the rate of growth in the root tip (Rønnike 1957 b).

In the following, the results of experiments on roots preliminarily germinated, partly in damp air between filter paper, partly in washed "Nodampoff" sphagnum with 300 per cent water, will be accounted for. The latter medium has been chosen because it is essentially of the same type as that used by Macht. The water content of 300 per cent has been chosen because it gives the optimum rate of growth, also during the later growth in nutrient solutions. Roots having the greatest sensitivity are thus obtained (Rønnike, 1958).

In investigations of the phytotoxicity of sera according to Macht, the growth of lupine roots in a 1 per cent solution of serum in Shive's " $R_5S_2$ "-solution diluted with equal amounts of water is used. Shive's solution is a nutrient medium consisting of three different salts, *viz.*,  $Ca(NO_3)_2$ ,  $MgSO_4$ , and  $KH_2PO_4$  (Shive, 1915). The addition of Fe, as used by Shive, was not adopted, however. Diluted Shive's solution has an osmotic pressure of about 1.3 atm. The sera solutions are prepared in the way that three ml serum is added to 297 ml of diluted Shive's solution (in the following briefly: "Shive's solution").

### STATISTICAL TREATMENT OF EXPERIMENTAL RESULTS

#### a. *Intraexperiment Variation.*

As demonstrated by the author (Rønnike 1958), the elongation of roots in nutrient solutions is dependent on the initial length of roots with the same period of preliminary growth. As

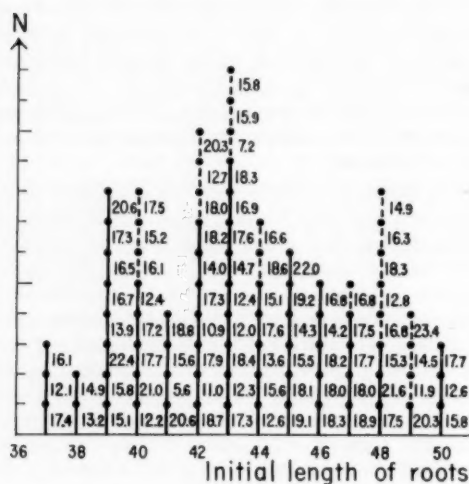


Fig. 3 a.

mentioned previously, the initial length is to be understood as the length of the root immediately before the transfer to the experimental solutions.

In order to investigate how great a variability this experimental factor will bring about in the individual experiment, the distribution of chronologically listed initial lengths of roots grown in Shive's solution and in one per cent serum solution, respectively, in one and the same experiment, has been shown in two diagrams (Fig. 3 a and 3 b). As suggested by Macht, initial lengths between 35 and 50 mm have been chosen. The roots shown by a dotted line are those which, in the first instance, were left out from the calculations, so that, within each initial length, ex-

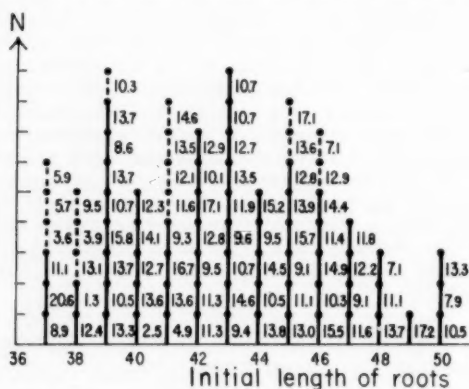


Fig. 3 b.

As Fig. 3 a. Initial lengths of 80 roots, placed in serum solution for further growth. The roots represented by a dotted line in Fig. 3 a and 3 b are such as are left out from the calculations.

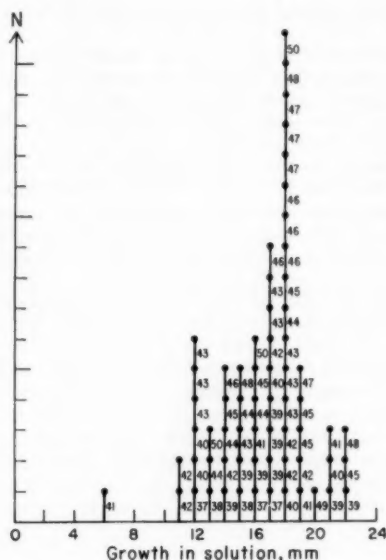


Fig. 4 a.

Diagram showing the distribution of the elongational increments of adjusted numbers of roots in Shive's solution. Beside each unit the corresponding initial length is indicated.

actly the same number of roots represent the Shive solution as well as the serum solution. The omission was, of course, done quite at random, the roots plotted at the top of the diagrams being discarded first.

Fig. 4 a and 4 b are diagrams corresponding to 3 a and 3 b representing the elongational increments in the Shive solutions and the serum solutions. According to these two diagrams, comprising 65 roots in each solution and with exactly

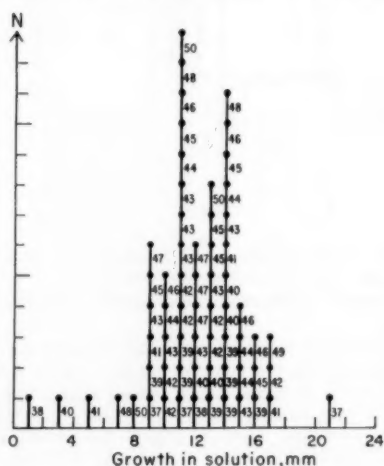


Fig. 4 b.

As Fig. 4 a. Distribution of elongational increments of adjusted numbers of roots in serum solution.

the same number of roots within each initial length, the average increments amount to  $16.3 \pm 0.8$  and  $11.9 \pm 0.8$  mm, respectively, in the Shive solution and in the serum solution. The index of elongation for the serum solution is  $73 \pm 6$ . (In this paper the confidence intervals are always 95 per cent intervals, see later). Calculation of the index value direct from the experimental tables covering the total number of roots, but different numbers within each initial length, gives  $71 \pm 6$ , and if the values are plotted in a probit

diagram (Fig. 5), where the numbers of roots are counted on a logarithmic scale, an index of  $78 \pm 6$  is arrived at. Accordingly, the above-mentioned uncorrected index of  $71 \pm 6$  is within the limits of confidence intervals identical with both of the other, somewhat different, calculated values. The fact that there is exactly the same number of roots with the same initial length in each solution has thus not shown any influence on the results.

The major cause by far of the lack of influence is, no doubt, that the variation of the initial lengths is kept within a narrow range (35—50 mm). In the subsequent work the limits to the variation of the initial lengths were further narrowed, so that, with a few exceptions, root lengths between 40 and 50 mm only were used, which made a calculation corrected in respect of the initial-length factor still less necessary. In the exceptional cases mentioned, initial root lengths between 42 and 52 mm or, say, 45 and 55 mm had been chosen.

With the given, standardised technique, the root increment in Shive's solution is normally distributed with an average standard deviation  $s$  of about 3.1 mm (determined as the average of 41 calculated cases).

The deviation (standard error) of an arithmetic mean value depends, of course, on the number of roots used in the determination. When 40 roots

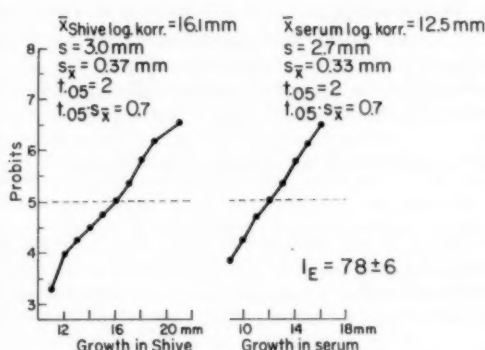


Fig. 5.

Probit diagram of the increments shown in Fig. 4 a and 4 b. The numbers of roots (the ordinates in Fig. 4 a and 4 b) are calculated on a logarithmic scale.

Table 1.

The intra-experiment variation of  $I_E$  (Relative growth) on two dates of sera from different probands. Growth in Shive- and sera-solutions in 24 hours at  $15^\circ \text{C}$ . (Standard technique: 60 hours' preliminary growth at  $20^\circ \text{C}$ . in washed sphagnum with 300 % water).

Dates	Experimental solutions	Number of roots (n)	Average initial length mm	$E_{\text{Shive or } E_{\text{serum}}}$ $\pm t.05 s_{\bar{x}}$ mm	Relative growth $I_E$ $\pm t.05 s_{\bar{x}}$
21—22/11 1957	Shive	40	43.7	$18.7 \pm 1.3$	$100 \pm 7$
	Serum I	40	44.3	$8.2 \pm 0.9$	$44 \pm 6$
	" II	40	44.0	$12.0 \pm 1.2$	$64 \pm 8$
	" III	38	46.3	$11.8 \pm 1.7$	$63 \pm 10$
	" IV	38	46.1	$11.9 \pm 1.3$	$64 \pm 8$
	" V	38	45.2	$13.1 \pm 1.0$	$70 \pm 7$
	" VI	38	45.5	$11.8 \pm 1.2$	$63 \pm 8$
	" VII	40	45.8	$12.2 \pm 1.2$	$65 \pm 8$
23—24/1 1958	" VIII	38	45.1	$10.3 \pm 1.0$	$55 \pm 7$
	Shive	40	45.6	$20.0 \pm 0.9$	$100 \pm 5$
	Serum A	40	44.2	$13.7 \pm 0.8$	$69 \pm 5$
	" B	40	44.2	$14.3 \pm 0.8$	$72 \pm 5$
	" C	40	45.1	$8.4 \pm 0.7$	$42 \pm 4$
	" D	40	44.7	$10.0 \pm 0.6$	$50 \pm 4$
	" E	40	46.0	$13.0 \pm 0.8$	$65 \pm 5$
	" F	40	45.6	$6.5 \pm 0.8$	$33 \pm 4$
	" G	40	45.2	$9.7 \pm 0.5$	$49 \pm 4$
	" H	40	45.7	$3.8 \pm 0.5$	$19 \pm 3$
	" I	40	45.0	$15.6 \pm 1.1$	$78 \pm 7$
	" J	40	45.3	$5.6 \pm 0.8$	$28 \pm 4$

are used, as is the rule, the standard error  $s_{\bar{x}}$  is equal to  $\frac{3.1}{\sqrt{40}} = 0.49$ . Multiplication of this quantity by 2.03 ( $= t_{.05}$ , S n e d e c o r 1956) gives the 95 per cent confidence limits for a given mean increment in Shive's solution. Similar considerations apply to growth in the sera solutions.

In each experiment the mean increment value in Shive's solution is put at 100, and the corresponding  $\pm$  value becomes equal to 6 determined as the average of forty-one calculated cases (two extreme values of 3 and 13, respectively).

The confidence intervals for a serum index determination are calculated from the formula

$$100 \times \frac{A}{B} \sqrt{\frac{a^2}{A^2} + \frac{b^2}{B^2}}, \text{ where}$$

$A$  is the increment in serum with  $a$  as the ancillary  $t_{.05} s_{\bar{x}}$ , and  $B$  is the increment in Shive's solution with  $b$  as the ancillary  $t_{.05} s_{\bar{x}}$  (modified according to Yule & Kendall 1940, p. 299). Within the same experiment, and with at least 36 roots in Shive's solution, as well as in each of the serum solutions, a 95 per cent safe  $\pm$  value of 5 (determined as the average of 121 calculated cases (two extreme values of 12 index units and two of 2)) is obtained.

If the difference between two index determinations within the same experiment  $\geq$  about 8–10 index units, there will, accordingly, be  $\geq$  95 per cent probability of such a difference being real in this single experiment.

By way of an example, Table 1 shows in detail the results of two experiments with sera from different persons (denoted I–VIII and A–J on November 21 & 22, 1957, and January 23 & 24, 1958, respectively). From this Table it is plain that in the individual experiment sera from different probands may have different phytotoxic properties. In the experiment on November 21 & 22, 1957, serum I is, for instance, more toxic than all the other sera measured in the experiment.

#### b. Dependence of Serum Toxicity on the Variability of the Comparison Value.

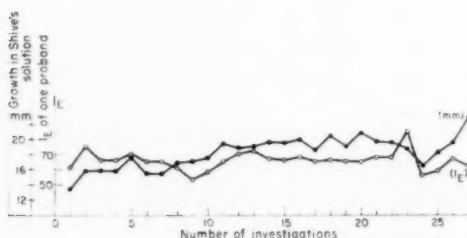


Fig. 6.

Curve showing the total number of  $I_E$  measured for one and the same proband with relevant elongational increments in Shive's solution. No correlation between  $I_E$  and the elongational increment in Shive's solution.

Fig. 6 shows a curve representing the variability of the comparison value with all the index determinations made for sera from one and the same proband. The coefficient of correlation  $r$  of these two curves is 0.168, and the probability of this value being nil is only about 60 per cent; the conclusion must then be that there is no dependence between the quantity of the comparison value and that of the index. It is therefore justifiable to compare index values from different experiments (cf. also A b e r g, 1954).

#### c. Interexperiment and Interproband Variation.

A single index determination (and, incidentally, any laboratorial determination in the clinic which can be expressed numerically) can be denoted by the symbol  $(X_{ijk})$ . According to S n e d e c o r, this symbol can be taken to be composed of a sum of several components, so that

$$X_{ijk} = \mu + A_i + B_j + (AB)_{ij} + \epsilon_{ijk}$$

where  $\mu$  is the theoretical mean of all index determinations.  $A_i$  is to be understood as a divergence therefrom, characteristic of the individual proband and with the proband standard deviation  $\sigma_A$ .  $B_j$  is to be regarded as a divergence characteristic of the divergence from experiment to experiment for sera from the same proband. This divergence has a standard deviation which may be denoted by  $\sigma_B$  ("the interexperiment variation").  $(AB)_{ij}$  is an "interaction component" which is contingent on the fact that the differences between indices from the same probands do not take a "synchronous" course from experiment to experiment. This component, too, has its particular standard deviation ( $\sigma_{AB}$ ).  $\epsilon_{ijk}$  is a variable component consisting of a normal distribution with the mean value 0 and the standard deviation  $\sigma$  corresponding to that of the individual index determination (the "intraexperiment variation" which, as mentioned above, has been calculated to be five as the average of 121 calculated cases). Consequently, according to the definition,  $\Sigma A = \Sigma B = \Sigma AB = 0$  ( $i$  being the symbol for the number of probands,  $j$  for the number of experiments, and  $k$  for the number of index determinations, S n e d e c o r, Table 12. 5. 4, 1956).

In Fig 7 an attempt has been made to sketch out the various parameters. The proband distribution is shown by the dot diagram to the left of the ordinate, each proband being represented by one unit. If a proband has been examined by repeated determinations, the average value has been figured on. As previously mentioned, the standard deviation for this distribution is  $\sigma_A$ . The interexperiment variation for sera from the individual probands is shown in the central and right-hand graphs as the large deflections of the curves about imagined horizontal axes. As previously mentioned, the standard deviation for this variation is  $\sigma_B$ . Finally, we have the intraexperiment varia-



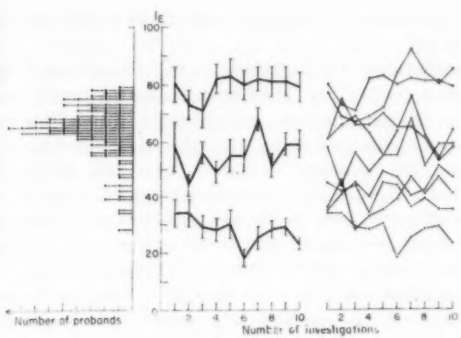


Fig. 7.

Left-hand graph: Diagram showing the distribution of  $I_E$  for 109 probands. Average of total number =  $6\frac{1}{2} \pm 2$  with an interproband standard deviation of 10.

Central graph:  $I_E$  for three probands each examined by ten determinations. For each  $I_E$ , 95 per cent confidence intervals are indicated, corresponding to the lengths of the vertical transverse lines on the curves.

Right-hand graph:  $I_E$  for nine probands, each examined by ten determinations. A considerable overlapping of the results is often seen.

tion, which is shown by the vertical transversal lines on the three curves in the central graph (the standard deviation  $\sigma$ ).

EXPERIMENTS ON ROOTS OF LUPINUS ALBUS GROWN PRELIMINARILY IN SPHAGNUM

Fig. 7 shows the results of 72 experiments made with seeds grown preliminarily for 60 hours at 20° C in washed sphagnum containing 300 per cent water (standard technique).

471 growth determinations have been made, each comprising 42 roots on an average. 71 out of the 471 determinations are comparison values or Shive-growth determinations, while 400 are growth determinations on sera from, altogether, 109 probands. The total number of sphagnum roots is 19,528, each of which has been measured twice, giving, altogether, 39,056 single measurements. In the Shive-growth investigations a total root elongation of 56,010.5 mm has been measured, and in the sera-growth determinations 176,566.0 mm (the roots were measured within an accuracy of  $\frac{1}{10}$  mm). The average elongation of the roots in Shive's solution is  $18.5 \pm 0.5$  mm ( $s = 2$  mm) (extreme values: one of 13.4 mm and one of 22.9 mm). In Fig. 7 the interexperiment variation for nine probands has been plotted. It will be seen that in three typical, selected cases in the central graph there is a distinct difference in the sera toxicity when the determinations are the results of several experiments. In the majority of cases by far, the values overlap from experi-

Table 2.

The inter-experiment variation of  $I_E$  (Relative growth) from sera of 2 probands. Growth in Shive- and sera-solutions in 24 hours at 15° C. (Standard technique: 60 hours' preliminary growth at 20° C in washed sphagnum with 300 % water).

Dates	Average initial length mm	Number of roots (n)	$E_{Shive}$ $\pm t \cdot 05 \sigma_x$ mm	Relative growth $\pm t \cdot 05 \sigma_x$	Average initial length mm	Number of roots (n)	$E_{serum}$ $\pm t \cdot 05 \sigma_x$ mm	Relative growth $I_E$ $\pm t \cdot 05 \sigma_x$
Shive's solution					Serum from one proband (O <sup>h</sup> B.R.)			
28-29/11 1957	44.5	40	17.5 $\pm$ 1.3	100 $\pm$ 7	44.4	40	7.9 $\pm$ 0.9	45 $\pm$ 5
5-6/12 1957	44.7	40	19.6 $\pm$ 0.9	" $\pm$ 5	45.2	40	8.2 $\pm$ 0.6	42 $\pm$ 4
9-10/12 1957	45.1	40	22.9 $\pm$ 0.9	" $\pm$ 4	43.8	40	10.0 $\pm$ 0.8	44 $\pm$ 4
12-13/12 1957	43.9	40	21.8 $\pm$ 0.9	" $\pm$ 4	46.1	40	7.4 $\pm$ 0.6	34 $\pm$ 3
16-17/12 1957	44.4	40	19.2 $\pm$ 1.3	" $\pm$ 7	44.6	40	8.6 $\pm$ 1.0	45 $\pm$ 6
19-20/12 1957	43.1	36	20.8 $\pm$ 1.0	" $\pm$ 5	43.4	36	9.2 $\pm$ 0.8	44 $\pm$ 5
2-3/1 1958	44.6	40	18.0 $\pm$ 1.5	" $\pm$ 8	44.0	40	6.5 $\pm$ 0.7	36 $\pm$ 5
3-4/2 1958	48.3	40	18.1 $\pm$ 0.7	" $\pm$ 4	48.9	38	7.1 $\pm$ 0.5	39 $\pm$ 2
6-7/2 1958	46.2	40	19.5 $\pm$ 0.7	" $\pm$ 4	47.4	40	6.8 $\pm$ 0.7	35 $\pm$ 3
17-18/2 1958	43.8	40	19.2 $\pm$ 0.9	" $\pm$ 5	45.0	40	6.8 $\pm$ 0.6	35 $\pm$ 4
Shive's solution					Serum from another proband (O <sup>h</sup> S.)			
31/10-1/11 1957	45.6	40	16.4 $\pm$ 1.9	100 $\pm$ 12	44.9	40	10.0 $\pm$ 1.5	61 $\pm$ 12
7-8/11 1957	45.8	40	15.9 $\pm$ 1.3	" $\pm$ 8	45.8	40	10.5 $\pm$ 1.5	66 $\pm$ 11
14-15/11 1957	44.6	40	15.6 $\pm$ 1.7	" $\pm$ 11	45.7	40	10.8 $\pm$ 1.5	69 $\pm$ 12
21-22/11 1957	43.7	40	18.7 $\pm$ 1.3	" $\pm$ 7	45.5	38	11.8 $\pm$ 1.2	63 $\pm$ 8
28-29/11 1957	44.5	40	17.5 $\pm$ 1.3	" $\pm$ 7	44.8	40	9.7 $\pm$ 1.3	55 $\pm$ 8
12-13/12 1957	43.9	40	21.8 $\pm$ 0.9	" $\pm$ 4	44.6	40	14.0 $\pm$ 0.9	64 $\pm$ 5
16-17/12 1957	44.4	40	19.2 $\pm$ 1.3	" $\pm$ 7	44.7	40	14.5 $\pm$ 1.3	76 $\pm$ 9
2-3/1 1958	44.6	40	18.0 $\pm$ 1.5	" $\pm$ 8	44.2	40	11.1 $\pm$ 1.1	62 $\pm$ 8
6-7/1 1958	44.7	40	22.0 $\pm$ 0.9	" $\pm$ 4	44.4	36	11.8 $\pm$ 1.0	54 $\pm$ 5
9-10/1 1958	43.8	40	19.2 $\pm$ 1.0	" $\pm$ 5	45.8	40	12.2 $\pm$ 0.9	64 $\pm$ 6

ment to experiment, as will appear from the graph on the extreme right in Fig. 7.

If in such cases one wants to decide whether there is any difference between the toxicity of the various probands, a larger number of determinations will have to be made and, in addition, more intricate methods of calculation will have to be used (Snedecor, p. 343: The significance for differences between index determinations from different probands in the F analysis has to be measured by the variance for AB in the denominator and not by  $\sigma^2$ ).

The interexperiment standard deviation  $\sigma_B$ , determined in 39 probands examined by means of five or more index determinations (altogether 319) is eight. This means that *index for the same proband may fluctuate by up to 32 index units*. (Two extreme single values in the indices for two different probands have, as mentioned previously, been found to be 18 and 92). Table 2, containing the results of experiments on several samples of sera from two probands, is an example of the interexperiment variation.

As previously mentioned, the intraexperiment standard deviation  $\sigma$  in Shive index (which is always 100) has been found to be  $\pm 6$  on an average, and  $\pm 5$  in sera.

From the diagram in the left-hand side of Fig. 7 the average index for 109 probands is found to be  $64 \pm 2$  with an interproband standard deviation ( $\sigma_A$ ) of 10. Consequently, 95 per cent of all probands have indices within the range of 44 and 84. (99 per cent of all probands have indices within 37 and 91). The majority by far of probands with indices below 50 have been tested by not less than six determinations.

#### EXPERIMENTS WITH ROOTS GROWN PRELIMINARILY IN DAMP AIR IN FILTER-PAPER TUBES

In the dry state, the *Lupinus albus* seeds which were used for experiments with seeds grown preliminarily in damp air in specially designed moistened filter-paper tubes had a somewhat larger diameter than the seeds grown in sphagnum (13–15 mm as against 9–11 mm).

The investigations of these roots comprise 41 experiments with, altogether, 85 growth determinations, *viz.*, 41 Shive determinations and 44 sera determinations. In these determinations, altogether 6,627 roots were used, out of which 3,314 were grown in Shive's solution and 3,313 in sera solutions. The average number of roots per growth determination was 78 (*i. e.* essentially more than used in the experiments with seeds germinated preliminarily in sphagnum). The aggregate root elongation was 84,764.2 mm, out of which 50,461.9 mm in Shive's solution and 34,302.3 mm in the sera solutions. The average root elongation in Shive's solution with these filter-paper germinated seeds was  $15.1 \pm 0.6$  mm

( $s = 2$  mm). Extremes: one of 12.0 and one of 18.8 mm.

Altogether 17 probands were tested, and the average index for these was found to be  $72 \pm 4$  ( $s = 7$  index units). Accordingly, this value has to be compared with the abovenamed  $64 \pm 2$  being the average value for the 109 probands tested by means of seeds grown preliminarily in sphagnum. There is thus a significant difference between the sensitivity of roots used in the two investigation series. Whether the difference is contingent on the size of the seeds or on the nature of the preliminary growth medium, has not been investigated.

The varying degree of sensitivity found in all probands as a whole has also been conspicuous in the investigation of sera from two probands tested with both types of roots.

One of these probands ( $\delta$  F.R.) was tested ten times with filter-paper germinated seeds, the average index being found to be  $75 \pm 3$  ( $s = 5$ ). This proband was also tested seven times with sphagnum-germinated seeds. The average index found in this case was  $64 \pm 3$  ( $s = 4$ ).

Thus, the index determinations for this proband corresponded in both cases to the average values for the total number of probands tested with the two types of roots (72 and 64).

The other proband ( $\eta$  B.R.) was tested 11 times by means of filter-paper germinated roots. The average index was found to be  $59 \pm 6$  ( $s = 8$ ). With the sphagnum-germinated seeds she was tested 10 times, and in this case an average index of  $39 \pm 5$  was found ( $s = 6$ ).

Thus, the index determinations for this proband corresponded in both cases to a level which was low in relation to the average level for the total number of probands tested with the two types of roots. The sensitivity to the extratoxic serum which this second proband displayed seemed, however, to be greater in the sphagnum-germinated roots than in those germinated in filter paper ( $64-39 > 73-59$ ).

#### THE PROBAND MATERIAL

The 126 probands were partly hospital patients suffering primarily from surgical diseases, and partly people belonging to the medical and nursing personnel of the hospital. Sera from children were not investigated.

Fifty-six men with 183 index determinations and fifty-three women with 217 index determinations were tested with seeds grown preliminarily in sphagnum. With seeds grown preliminarily in filter-paper tubes nine men with 25 index determinations and eight women with 19 index determinations were tested. Two probands, *viz.* one male and one female, are common to the two experimental groups mentioned.

Thirty probands (8 men and 22 women) were selected from among the medical and nursing staff. Out of the 22 women, 19 were selected

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from among healthy, regularly menstruating, young women. Of these, 17 were tested by repeated determinations, made regularly throughout at least two catamenial periods.

Fifty-eight probands (46 men and 12 women) were selected from among the patients in a surgical department with "purely" surgical diseases (fractures, hernias, kidney and gallstone diseases, abscesses, etc.). From the same department 25 patients (4 men and 21 women) suffering from cancerous diseases were selected (18 women with cancer mammae — 13 with and 5 without verified metastases, two women with metastatic peritoneal cancer (partly *c. coli*, partly *c. ovarii*); one woman had renal hypernephroma. The four men suffering from cancerous diseases had metastatic renal cancer, metastatic melanotic sarcoma, metastatic rectal cancer, and testicular cancer, respectively). Concurrently with the root-growth investigations, many of the cancer patients received an intensive X-ray treatment.

Additionally, the proband material comprises three men from psychiatric departments with manifest psychoses, two pregnant, healthy women, one woman with pemphigus vulgaris (well treated with Cortisone), three untreated patients with newly diagnosticised pernicious anaemia and one patient with well treated pernicious anaemia, one patient with pronounced thrombopenia essentialis, and one patient with polyglobulia.

#### COMPARISON WITH MACHT'S EXPERIMENTAL RESULTS

As will appear from what has been stated previously in this paper, Macht's indication of the standard index value as 70—80 agrees very well with the mean value for the seeds germinated in filter-paper tubes, but not with the mean value for the seeds germinated in sphagnum which, as mentioned in the foregoing, is  $64 \pm 2$ . Like Upjohn et al., the author has been unable to confirm the results of Macht's investigations as regards the toxicity of sera from patients who have been subjected to X-ray therapy, nor from patients suffering from psychoses, pernicious anaemia or a single patient with pemphigus. Furthermore, contrary to what was the case in Macht's investigations, these experiments have disclosed that healthy probands may have very low indices, i.e., a high toxicity in their blood.

In a single proband (♀ A. C., Fig. 8) an additional toxic factor seems to be present in the blood, apparently synchronously with the menstruation. The correlation found in this proband is, however, no doubt accidental seeing that in all the other, normally menstruating, women examined no increased toxicity occurred in the blood during the menstruation. According to Macht, increased toxicity should always be found during catamenia.

The greatest toxicity in the blood was found in a 65-year-old woman with *c. mammae dxt.*

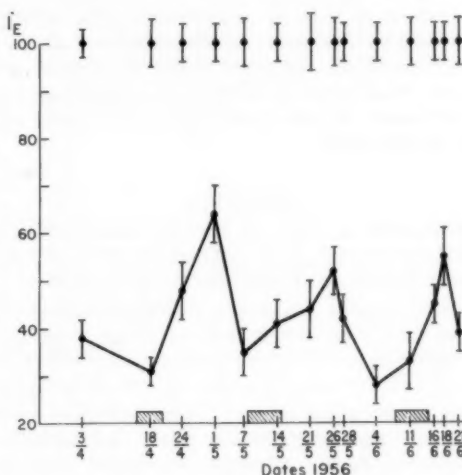


Fig. 8.

Fourteen consecutive  $I_E$  determinations in a 28-year-old, healthy woman (♀ A. C.). Shaded areas above the abscissa indicate the lengths and times of three catamenial periods. At the top of the graph the 95 per cent confidence intervals for the growth in Shive's solution are indicated.

ulc. inoperabilis without distant metastases. The average of ten examinations during two years between the first and the last examinations, showed an  $I_E$  of  $28 \pm 4$ . The tumour in this patient has been manifest for 15 years. 17 other patients with mammary cancer did not display toxicity deviation from the average; especially, two patients\*) of the same type as the above-named patient with the maximum toxicity appeared to have normal indices.

In conclusion, human serum can be divided into four groups, according to the degree of its inhibitory effect on the growth of plant roots, viz.:

- 1) Serum with a slight toxicity (phytotoxic index  $I_E > 70$ ).
- 2) Serum with an average toxicity ( $70 > I_E > 50$ ).
- 3) Serum with a heavy toxicity ( $50 > I_E > 37$ ).
- 4) Serum with the maximum toxicity ( $I_E < 37$ ).

Ninety-nine per cent of the sera of all probands have phytotoxic indices between 37 and 92.

#### Addendum.

In a later paper a number of characteristics of the plant-root growth-inhibitory properties of serum will be accounted for.

\*) For assistance in selecting these patients the author is indebted to Professor Jens Nielsen, The Radium Centre of Copenhagen.

It may be said already now that the inhibition of serum is due to an oligodynamically acting, thermostable, dialysable factor (factors) in the blood, partially combined with the serum albumins. From these it is liberated partially under the influence of inorganic ions and totally by heat denaturation.

#### SUMMARY

An account is given of previous investigations on the occurrence in the animal kingdom of substances possessing a plant-hormonal character.

In the present paper the inhibitory effect of human serum on the growth of plant roots, investigated by means of the lupine root method of Macht-Livingston, is accounted for.

The intraexperiment and interexperiment variations are accounted for in detail, and likewise the interproband variation examined in, altogether, 126 probands.

Human serum can consequently be divided into four classes according to the grade of its toxicity to the growth of plant roots.

It is further demonstrated that lupine seeds grown preliminarily in sphagnum and having diameters between 9 and 11 mm have a sensitivity to serum differing from that of other lupine seeds (with diameters of 13 to 15 mm) grown in damp air in filter-paper tubes.

So far, it has been impossible to establish any relationship between the inhibitory effect of serum on the growth of lupine roots and any disease.

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